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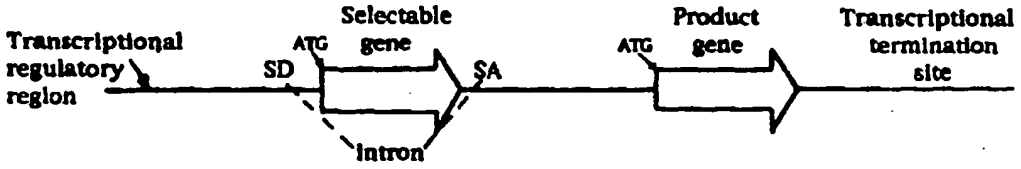

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., **264(24)**: 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (ΔGT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [ΔGT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ΔGT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	Na'K'-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5' end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3' end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerasechain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^-$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR$^-$ cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT 1000

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA 3800

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

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GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC 3300

GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT 4350

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC 600

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG 5500

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TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5750

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AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 2050

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

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GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC 2300

ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT 2850

TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA 2900

GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

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CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT 3150

TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC 3200

CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC 3250

CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG 3300

CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA 3350

CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA 3400

AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC 3450

TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG 3500

CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG 3550

CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG 3600

CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA 3650

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CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG 4400

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ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA 4750

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GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC 5000

TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC 5050

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GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA 5400

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ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT 5600

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AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 5900

GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 5950

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6000

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6050

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GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT 6250

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TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG 6600

CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG 6650

CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6700

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6750

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TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG 6900

CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC 6950

TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA 7000

GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG 7050

TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG 7100

CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA 7150

AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG 7200

CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

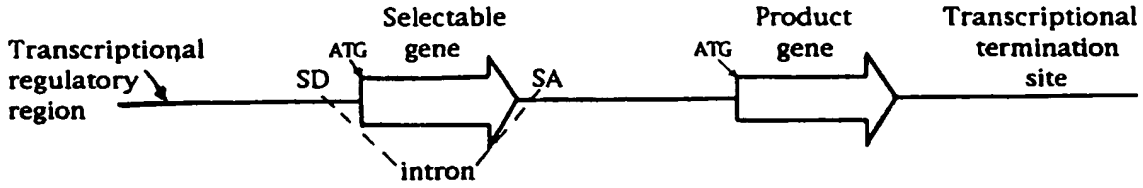
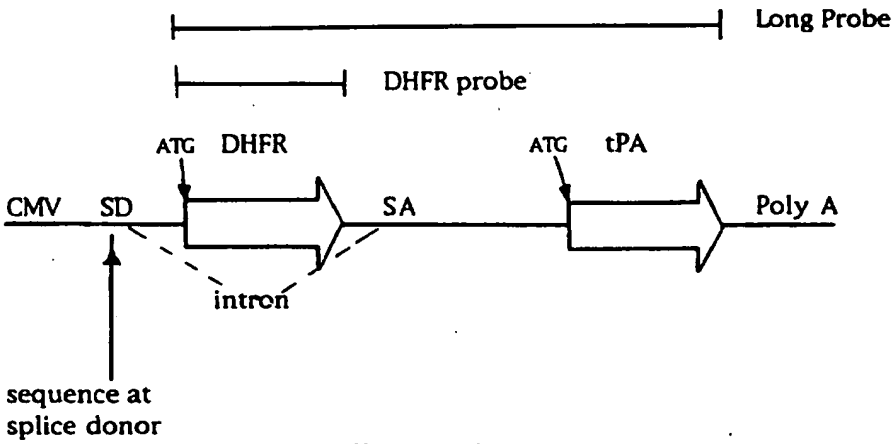
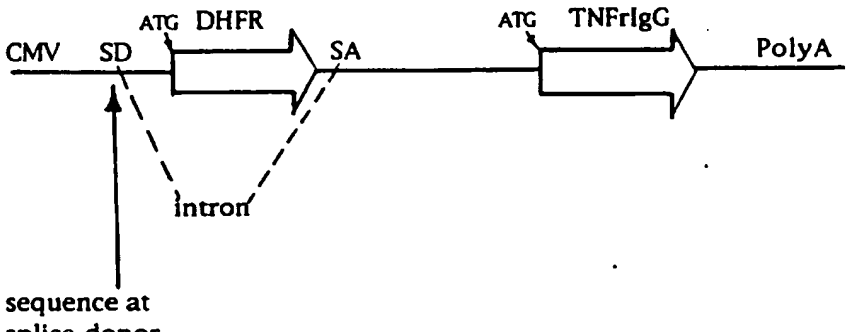


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

FIG. 1D

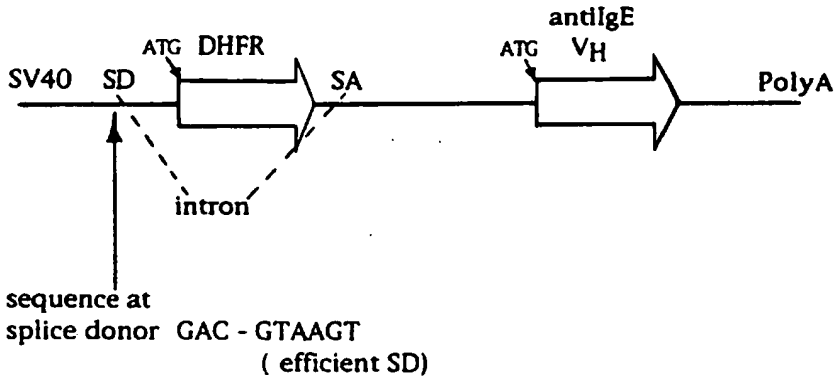

antiIgE
ATG DHFR
ATG V_H
SV40 SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

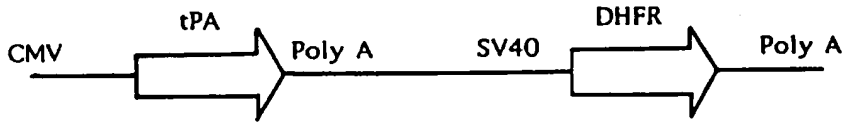

tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
      aluI
      sstI
      sacI
      hgiJII
      hgiAI/aspHI
      ecl136II
      bsp1286                                                                    thaI
      bsiHKAI                                                                    fnuDII/mvnI
      bmyI                        rmaI    tru9I                                  bstUI
      banII                       maeI    mseI                                   bsh1236I
    taqI                          speI    aseI/asnI/vspI                bslI     aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                    scrFI
                    mvaI
                    ecoRII
                    dsaV
                  aciI
               bglI bstNI                              maeII
              sau96I                                  hinlI/acyI
              haeIII/palI     aciI                    ahaII/bsaHI
              asuI  apyI[dcm+]                aciI    aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                     maeII
     hinlI/acyI                                                                 hinlI/acyI
     ahaII/bsaHI                                  rsaI                  rsaI    ahaII/bsaHI
     aatII                              bglI      csp6I          ndeI   csp6I   aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
       aciI                                                                     nlaIII
      bglI  dsaV                                                          styI
     sau96I bstNI           rsaI                              maeII              ncoI
     haeIII/palI            csp6I                       rsaI   snaBI           dsaI hphI  aciI
     asuI   apyI[dcm+]   bsrI  nlaIII                    csp6I  bsaAI           bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 3B

```
                                                                                                  maeII
                                                                                                 hinlI/acyI                nlaIV
                  rsaI                                 pleI                                      ahaII/bsaHI               hgiCI
                  csp6I                          aciI  hinfI                  bsmAI              aatII                     banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                                  aluI
                                                                                                                 sstI
                                                                                                                 sacI
                                                                                                                 hgiJII
                                                                                                                 hgiAI/aspHI
                                                                                                                 ecl136II
                                                                                                                 bsp1286
                                                                                                                 bsiHKAI
                                                                                                  rsaI           bmyI
                                  maeIII     aciI        hgaI        aciI        csp6I     mnlI                 banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                                   haeIII/palI
                                                                                                                  mcrI
                                                                                                                  eagI/xmaIII/eclXI
                                                                                                                  eaeI
                                                                                                                  cfrI
                                                                                                                 fnu4HI
                                                                                                                 aciI
                                                                                                                thaI
                                      esp3I                                                                      fnuDII/mvnI
                                 scrFI                                                  sau96I          sacII/sstII
                                 mvaI bsmAI                                                  avaII            nspBII
                                 ecoRII                                                 asuI             kspI  scrFI
                                 dsaV                                                  nlaIV             dsaI  nciI
                                 bstNI  hinlI/acyI                                        scrFI           bglI bslI  mspI
                                 apyI[dcm+]                                               nciI      sau3AI mnlI bstUI
                            sau3AI gsuI/bpmI                                              mspI      mboI/ndeII[dam-] hpaII
                            mboI/ndeII[dam-]                                    mboII     hpaII     dpnI[dam+] bsaJI dsaV
                            dpnI[dam+]  hgaI  fokI                              bpuAI     dsaV      dpnII[dam-] bsh1236I
                            dpnII[dam-] ahaII/bsaHI                    mnlI     bbsI      cauII     alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 3C

```
                tfiI
              aciI
             thaI hinfI
             fnuDII/mvnI                                  aciI                                                  fnu4HI
             bstUI                                      rsaI                                                    bbvI
             bsh1236I                                   csp6I   scfI                mnlI                        nspBII          taqI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT   aciI       nlaIII
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                                               thaI
                                                                                                               fnuDII/mvnI
                                                                                                               bstUI
                                                                                                               bsh1236I
                                                                                                             mluI
                                                                                  bsrBI                      aflIII      rsaI
                    pflMI                                   bsmAI                 aciI                xmnI            csp6I
    sfaNI           bslI                                    bsaI                mnlI   ddeI asp700              scaI
801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
    CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                                                  scrFI
                                                                                  mvaI
                                                                                  ecoRII
          eco57I                                                                  dsaV
        mboII                           tfiI                                      bstNI                                 tfiI          tru9I          tru9I
       earI/ksp632I                     hinfI                                     apyI[dcm+]                          mboII  taqI    mseI           mseI
     mnlI                             alwNI     hphI                           sexAI                        ddeI     hinfI     ahaIII/draI    aseI/asnI/vspI
901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
    TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                                  aluI
                                                  sstI
                                                  sacI
                                                  hgiJII
                                                  hgiAI/aspHI
                                                  ecl136II
                                                  bsp1286
                                                  bsiHKAI
                                                  bmyI                                                                 tru9I                  mspI
                                                  banII                                                              sfaNI  mseI                   hpaII
          ddeI                          bslI    mnlI                          bstXI       fokI                     aflII/bfrI                 bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

```
                                                                                  haeIII/palI
                                                                                haeI
                                                 scrFI          scrFI                                 nlaIII
                                                 mvaI                 mvaI                                      sau3AI
                                                 ecoRII         ecoRII                              mboI/ndeII[dam-]
                                                 dsaV    tfiI   dsaV    pleI                        dpnI[dam+]
           accI   nlaIII                         bstNI      nlaIII    bstNI      ddeI                           dpnII[dam-]
                                  mnlI           apyI[dcm+]    hinfI  apyI[dcm+]     hinfI      maeIII   alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                     mnlI
                                                                                 hinlI/acyI     scrFI
                                                                                 ahaII/bsaHI    mvaI
                                                                              scrFI        ecoRII
                                                                              mvaI    ecoNI     dsaV
                                                                              ecoRII     sau96I
                 maeII                                                        dsaV       avaII
               aflIII                                                         bstNI   bslI    asuI    mnlI
        apoI     maeIII                                                       apyI[dcm+]     mnlI   bstNI
                                                          mnlI              bsaJI hgaI       ddeI   apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                            ppu10I
                                                       sfaNI                                nsiI/avaIII
        sfaNI                    accI       mboII      mboII                     mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                 fnu4HI
       nlaIII                                    aciI
      styI                                      thaI
      ncoI                                      fnuDII/mvnI tru9I
      dsaI                        styI          bstUI        mseI                                        maeIII
      bsaJI                       bsaJI         bsh1236I   aseI/asnI/vspI                               hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 3E

```
                                                           scrFI
                                                           mvaI
                                                           ecoRII                                bspMI
                                                           dsaV                                scfI
                                                           bstNI                                     pstI
                                                           apyI[dcm+]      mnlI        aluI        bsgI                  tru9I
                                                         gsuI/bpmI         bsaJI       hindIII   fnu4HI                  mseI
      scfI         fokI                              maeIII      hincII/hindII      ddeI         bbvI             apoI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATTCGAACCC GACGTCCAGC GGCACTTAAA

                                                    hgiJII                                                scrFI
                                                    bsp1286                                               mvaI
                                                    bmyI              fnu4HI                              ecoRII
                                       sfaNI      mnlI                bbvI                 mboII          dsaV
                                      fokI     mboII  banII        fnu4HI                bpuAI            bstNI
        hgaI                        nlaIII     earI/ksp632I        bbvI                  bbsI             apyI[dcm+] nlaIII
1601 AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC
     TTCCCTGCGA CACTTCGTTA GTACCTACGT TACTTCTCTC CCGAGACGAC ACACGACGAC GACACACCTC GTCAGAAGCA AAGCGGGTCG GTCCTTTAGG

                                                      scfI
                                       sau3AI         pstI
                                       mboI/ndeII[dam-] bsgI                                                       hgiAI/aspHI
                               nlaIV   dpnI[dam+]   sau3AI                                                    hinPI bsp1286
                             mnlI    bstYI/xhoII    mboI/ndeII[dam-]                                        fnu4HI  bsiHKAI
          tfiI     mboII             bglII          dpnI[dam+]                                              bbvI    bmyI
          hinfI    earI/ksp632I dpnII[dam-]         dpnII[dam-]                                         nlaIII  hhaI/cfoI
1701 ATGCCCGATT CAGAAGAGGA GCCAGATCTT ACCAAGTGAT CTGCAGAGAT GAAAAAACGC AGATGATATA CCAGCAACAT CAGTCATGGC TGCGCCCTGT
     TACGGGCTAA GTCTTCTCCT CGGTCTAGAA TGGTTCACTA GACGTCTCTA CTTTTTTGCG TCTACTATAT GGTCGTTGTA GTCAGTACCG ACGCGGGACA

                   scrFI
                   nciI
                   mspI
                   hpaII                                             alwNI
                   dsaV                               bsp1286      ddeI                    fnu4HI   styI
       ddeI        cauII     sspI                     bmyI       draIII                    bbvI     bsaJI
1801 GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC
     CGAGTCTTCG TTGGCCCACC TTATAACGAC CACGTTGTCA CCGTCCCGTG TCACGGTGAG TCACGGACAG TTTTCAACGT CGCTCGGTTC CACAAAGTTG
```

FIG. 3F

```
                                                                                                              pmlI
                                                                                                              eco72I
                                                                                                          sau96I
                                                                                                          asuI maeII
                                                                                                      scrFI
           bspMI                                                                                      mvaI haeIII/palI
        nlaIV          sau96I                                                                         ecoRII
        hgiCI          haeIII/palI                                                                    dsaV
        banI           asuI   rsaI                      bsp1286                                       bstNI   bsaAI
       bsp1286        eco0109I/draII                    bmyI                                          bsaJI   bbrPI
       bmyI          alwNI    csp6I ddeI             bsrI      bcgI                                   apyI[dcm+]
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

                scrFI                                      hinPI
                mvaI                                             hhaI/cfoI
                ecoRII                                    nlaIV    hgiAI/aspHI
                dsaV                                      narI     bsp1286                thaI
                bstNI                   pmlI  hgiAI/aspHI       kasI     bsiHKAI                fnuDII/mvnI
                bsaJI                   eco72I                  hinlI/acyI                      bstUI sau96I
            sau96I                bsaAI bsp1286           hgiCI    bmyI                 hinPI   haeIII/palI
            avaII                 bbrPI bsiHKAI           haeII    apaLI/snoI           hhaI/cfoI
            asuI     sfaNI   scfI  bsp1286   bmyI    aciI       banI     alw44I/snoI      fnu4HI     asuI        bslI
          mnlI  apyI[dcm+] aluI    bmyI maeII      nspBII     ahaII/bsaHI     bsrI        bbvI bsh1236I        bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                             scrFI
                                             mvaI
                                             ecoRII
                                             dsaV                                  pleI        scrFI
                                             bstNI                                     bsmAI       mvaI
                                             bsaJI                               taqI[dam-]        ecoRII
                         hinlI/acyI          apyI[dcm+]                        sau3AI hinfI        dsaV
              aciI  haeIII/palI            haeIII/palI           scfI          mboI/ndeII[dam-]    bstNI
        nspBII     haeI  hgaI bstXI       sau96I         pstI           dpnI[dam+]        apyI[dcm+]     tru9I
     scfI aciI    mnlI   ahaII/bsaHI      asuI     nlaIV         bsgI          dpnII[dam-]      bsaJI    maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

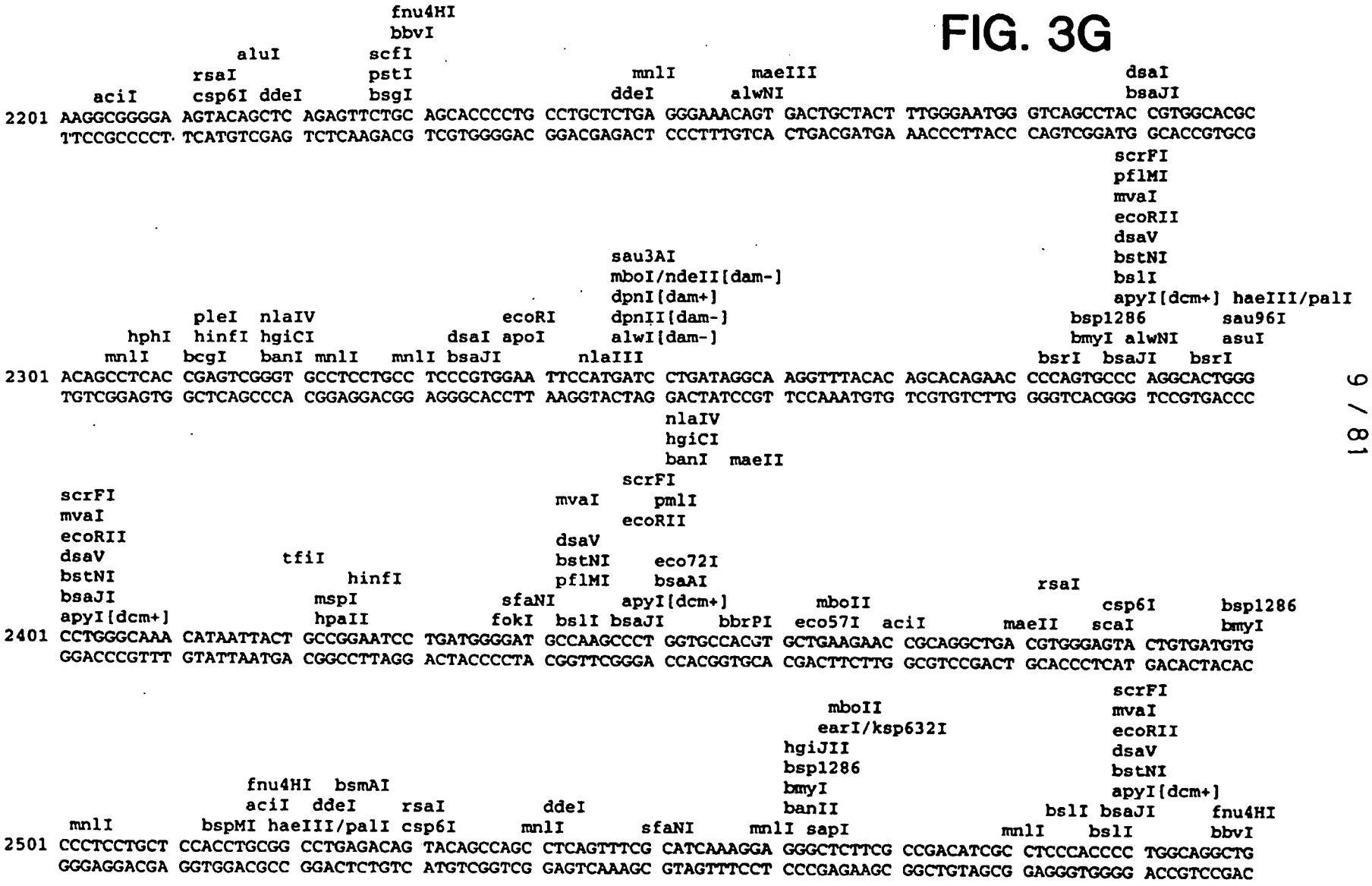


FIG. 3H

```
                                        mspI
                                        hpaII
                                        bslI                                                                                    scrFI
                                        scrFI                                                                                   mvaI
                                        nciI        nlaIV                                                                       ecoRII
                                        dsaV      aciI                                                                          dsaV
                                                                                                aluI        tfiI        aciI    bstNI
                bslI        mnlI        cauII   bsrBI     bslI aciI                             alwNI       hinfI       fnu4HI  apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                                        scrFI
                                                                                        mvaI
                                                                                        ecoRII
                                                                                        dsaV
                                                                                        bstNI
                                                                                        apyI[dcm+]
                                                                              scrFI     bslI
                                        sau3AI                                nciI      bsaJI
                                        mboI/ndeII[dam-]                      mspI    sau96I
                                        dpnI[dam+]                            hpaII   nlaIV
                                        dpnII[dam-]                           dsaV    avaII         mnlI
   mnlI     aciI     bslI       hphI[dam-]                                    cauII   asuI        mnlI        apoI        taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                                              scfI                                  scrFI
                                                                              pstI                                  mvaI
                                                                            fnu4HI                                  ecoRII
                                                                            bbvI pvuII                              dsaV
                                                                         fnu4HI fnu4HI                              bstNI                aciI
                                                                         bbvI   bbvI                          bsp1286      fnu4HI  sau96I
            ecoRI                                                      hinPI bsgI aluI       tfiI     nspBII  bsaJI     bbvI    avaII
            apoI taqI                                                  hhaI/cfoI nspBII      hinfI    aciI bmyI apyI[dcm+]       asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                                aluI
                                                               sstI
                                    pvuII                      sacI
                       mspI      scfI                          hgiJII
                       hpaII     pstI  fnu4HI                  hgiAI/aspHI
                      scrFI    bspMI   bbvI                    ecl136II                    haeIII/palI
                      nciI   sau96I nspBII                     bsp1286                    stuI
                      dsaV   avaII fnu4HI      bsrI            bsiHKAI                    haeI
                      cauII  asuI  bbvI    mspI                bmyI    mspI              mnlI
      draIII    bslI bsaJI aciI  bsgI aluI hpaII               banII   hpaII          nlaIII      bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                     fnu4HI
                                                   nspBII
        fnu4HI                                     aciI                                                 nlaIII
        aciI                          rsaI fokI   fnu4HI                   tru9I        hphI        nspI
      bsrBI eco57I mnlI  nlaIII       csp6I    alwNI bbvI                  mseI        maeIII       nspHI           gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                         sau96I
                         nlaIV
                         haeIII/palI
                         asuI
                        sau96I
                        nlaIV scrFI                                       scrFI
                        hgiJII                                                 mvaI
                        eco0109I/draII                  scrFI            ecoRII
                        bsp1286                         mvaI                   dsaV
                        bsp120I                         ecoRII           bstNI
                        bmyI  mvaI                      dsaV        sau96I
                        banII ecoRII          bstNI                 nlaIV                       aciI
                      aciI    dsaV            bsaJI                 haeIII/palI                fnu4HI
                   fnu4HI     bstNI        hinlI/acyI       tfiI      asuI apyI[dcm+]               haeIII/palI
                   aciI asuI  apyI[dcm+]   hgaI     apyI[dcm+]   eco0109I/draII                    eaeI
      bsmAI      bsrBI  apaI bsaJI         ahaII/bsaHI      hinfI   mnlI  bsaJI                    cfrI   nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

```
                                                        scrFI
                                                        nciI
                             scrFI                mspI
                             mvaI                       hpaII
                             ecoRII               dsaV
                             dsaV                 cauII
                             bstNI                     xmaI/pspAI
                             bsaJI                     smaI
                             apyI[dcm+]                scrFI
                        nlaIV                          nciI
                        eco0109I/draII           dsaV
                    aluI sau96I                  cauII     rsaI  styI maeIII                tfiI        nspI
                   pvuII asuI                          bsaJI     csp6I bsaJI             rmaI     hinfI       nspHI
           sfaNI   nspBII  haeIII/palI           fokI  avaI     bsp1407I   bstEII        maeI bsrI      maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                             mboII
                scrFI                            aciI earI/ksp632I
                mvaI                               sau3AI mnlI
                ecoRII                         mboI/ndeII[dam-]
                dsaV                           dpnI[dam+]             mboII
                bstNI            mnlI              dpnII[dam-]        eco57I
         bslI   apyI[dcm+]   pleI                  alwI[dam-]        mboII  bpuAI               hinPI
      mcrI maeIII            hinfI                bstYI/xhoII     mboII     bbsI                hhaI/cfoI      scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                        bsmAI                mboII
      gsuI/bpmI        aciI     aciI    bsaI  scfI     mnlI  earI/ksp632I                                 tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                    scrFI
                                                                  scrFI       mvaI
                                                                  mvaI bsmI         ecoRII
                                                                  ecoRII      dsaV
                                                                  dsaV        bstNI
                                      mboII                       bstNI             bsaJI
                                      bpuAI   bsmI     rmaI     gsuI/bpmI   mnlI   bsaJI                 ecoRI  taqI
                                      bbsI nlaIII      maeI mnlI apyI[dcm+]    mnlI apyI[dcm+]           apoI  claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
                styI
              aciI
             fnu4HI   sau96I
             bglI nlaIII
            sfiI ncoI haeIII/palI
            haeIII/palI                    aluI
           eaeI  dsaI asuI               fnu4HI
           cfrI  bsaJI                   bbvI          maeIII              sfaNI  apoI                                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                                sau3AI
                                                                mboI/ndeII[dam-]
                                                                dpnI[dam+]
                                                                dpnII[dam-]
                                                               pvuI/bspCI
                                                               mcrI
                                                              taqI[dam-]  tru9I
                                                            claI/bsp106[dam-]              styI
                                                           sau3AI          mseI        fnu4HI    haeI
                                                           mboI/ndeII[dam-]            bbvI  ncoI
                                                           dpnI[dam+] xmnI         hinPI    dsaI haeIII/palI
          rmaI                                             dpnII[dam-]    aseI/asnI/vspI    bsaJI
          maeI                                        nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII           mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                       rsaI                                                                    nlaIV
                       csp6I                                                                 scrFI
                      nlaIV                                                                  mvaI
                      kpnI                                                                   ecoRII
                      hgiCI                                                                  dsaV
                      banI                          aluI                                     bstNI
                      asp718     mnlI               pvuII                                    apyI[dcm+]
          mnlI        acc65I   ddeI aciI            nspBII                                 bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                                         nlaIV
          sfaNI                  scrFI         scrFI                                   sfaNI
        ppu10I                   mvaI                mvaI                                        ppu10I
        nsiI/avaIII              ecoRII        ecoRII                                nsiI/avaIII
       nlaIII                    dsaV          dsaV                                 nlaIII
      sphI                       bstNI               bstNI                                     sphI
      nspI                       apyI[dcm+]          apyI[dcm+]                                nspI
      nspHI                     sexAI               bsaJI                                      nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                     nlaIII
                                                                                     styI
                                                                                     ncoI
                                  aciI                                      bslI     dsaI
          aciI         aciI  fokI                aciI bsrI  aciI            aciI     bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                          rmaI
                                                                         styI
          aciI                                                           bsaJI
         fnu4HI                                                          blnI
        sfiI                                                             avrII
        haeIII/palI                                                    haeIII/palI             tru9I
    bsaJI bglI         ddeI                                            stuI                   hpaI
  haeIII/palI mnlI haeIII/palI                         mnlI            haeI                   hincII/hindII
 mnlI   mnlI   bsaJI  mnlI  aluI                     mnlI             mnlI maeI           aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                   scrFI
                                   mvaI
                                   ecoRII
   haeIII/palI                     dsaV
  eaeI                             bstNI                                                   aluI
  cfrI                      bsrI   apyI[dcm+]        tru9I       fnu4HI                   pvuII
 bsrI               maeII maeIII  bsaJI   maeIII     mseI        bbvI   fokI              nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

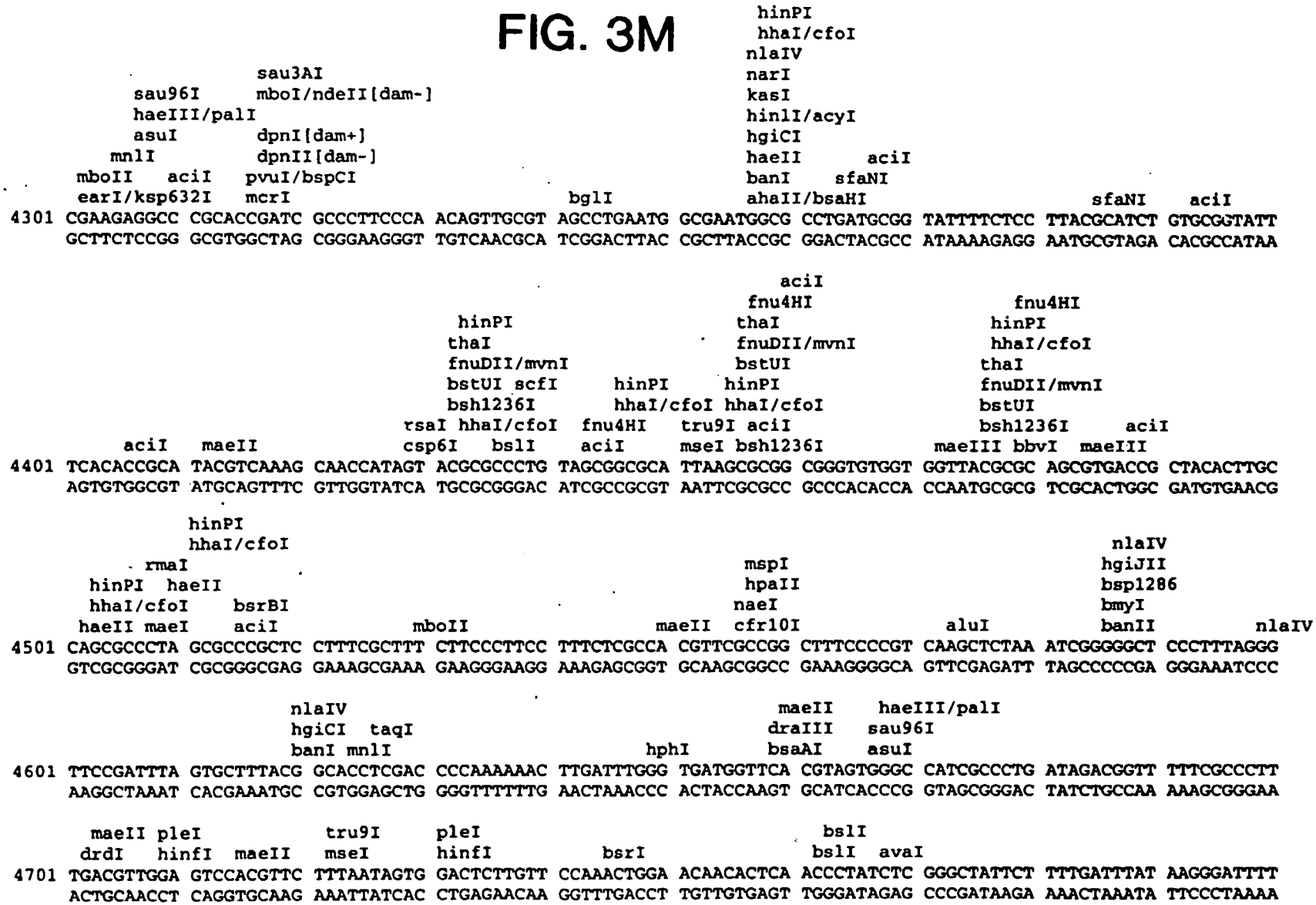

hinPI
hhaI/cfoI
nlaIV
sau3AI
narI
sau96I
mboI/ndeII[dam-]
kasI
haeIII/palI
hinlI/acyI
asuI
dpnI[dam+]
hgiCI
mnlI
dpnII[dam-]
haeII
aciI
mboII
aciI
pvuI/bspCI
banI
sfaNI
earI/ksp632I
mcrI
bglI
ahaII/bsaHI
sfaNI
aciI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA
aciI
fnu4HI
fnu4HI
hinPI
thaI
hinPI
fnuDII/mvnI
thaI
hhaI/cfoI
bstUI
thaI
fnuDII/mvnI
bstUI scfI
hinPI
hinPI
bstUI
bsh1236I
hhaI/cfoI
hhaI/cfoI
bsh1236I
aciI
rsaI
hhaI/cfoI
fnu4HI
tru9I
aciI
bsh1236I
maeIII
bbvI
maeIII
aciI
maeII
csp6I
bslI
aciI
mseI
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG
hinPI
hhaI/cfoI
nlaIV
rmaI
mspI
hgiJII
hinPI
haeII
hpaII
bsp1286
hhaI/cfoI
bsrBI
naeI
bmyI
haeII
maeI
aciI
mboII
maeII
cfr10I
aluI
banII
nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC
nlaIV
maeII
haeIII/palI
hgiCI
taqI
draIII
sau96I
banI
mnlI
hphI
bsaAI
asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA
maeII
pleI
tru9I
pleI
bslI
drdI
hinfI
maeII
mseI
hinfI
bsrI
bslI
avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA

FIG. 3N

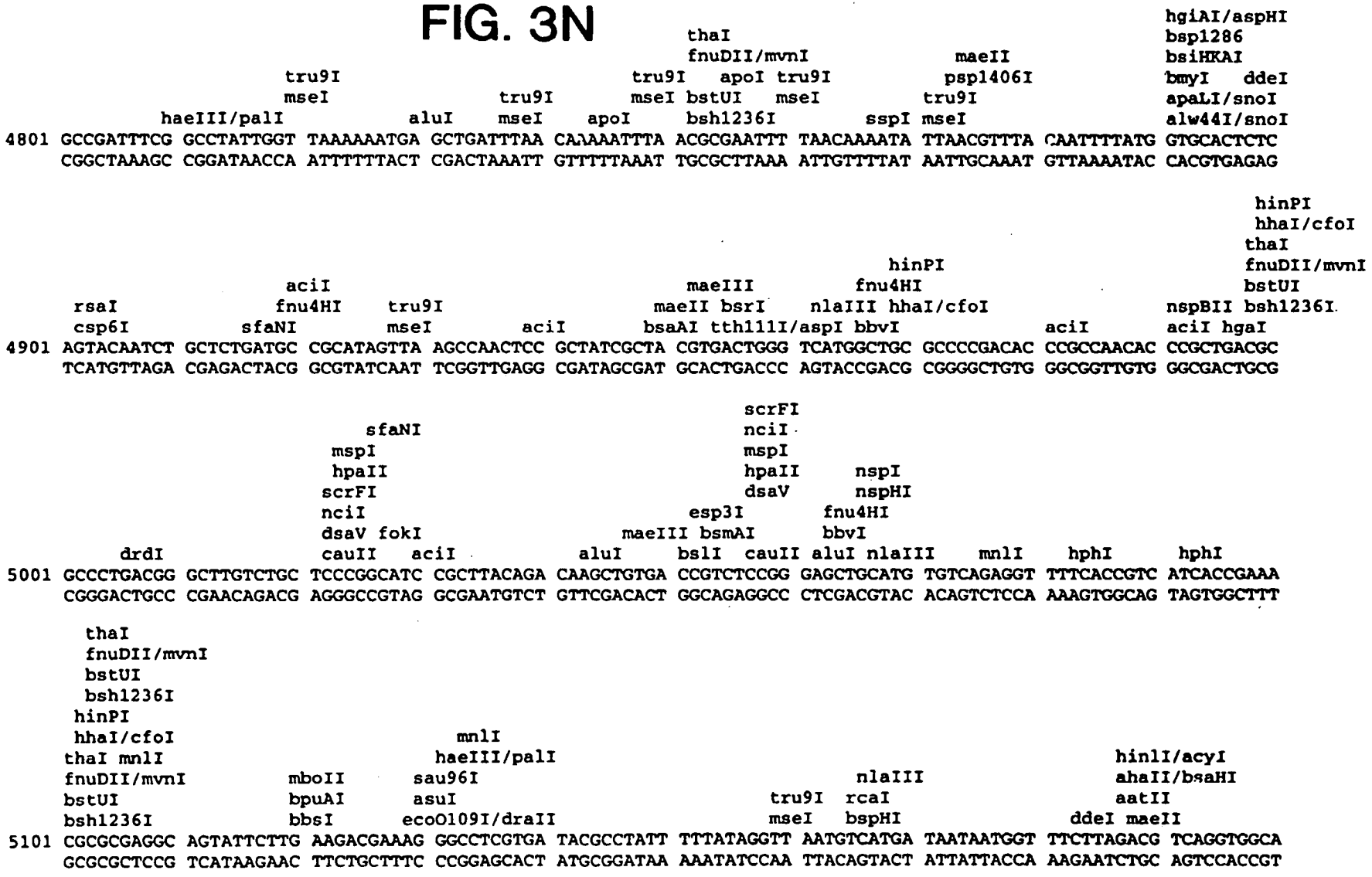

hgiAI/aspHI
bsp1286
bsiHKAI
bmyI ddeI
apaLI/snoI
alw44I/snoI
thaI
fnuDII/mvnI
maeII
psp1406I
tru9I
mseI
tru9I
apoI tru9I
tru9I
mseI bstUI mseI
haeIII/palI
aluI
mseI
apoI
bsh1236I
sspI mseI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
nspBII bsh1236I
aciI hgaI
hinPI
aciI
maeIII
fnu4HI
rsaI
fnu4HI
tru9I
maeII bsrI
nlaIII hhaI/cfoI
csp6I
sfaNI
mseI
aciI
bsaAI tth111I/aspI bbvI
aciI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG
scrFI
nciI
sfaNI
mspI
mspI
hpaII
hpaII
nspI
scrFI
dsaV
nspHI
nciI
esp3I
fnu4HI
dsaV fokI
maeIII bsmAI
bbvI
drdI
cauII aciI
aluI
bslI cauII aluI nlaIII
mnlI
hphI
hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
mnlI
thaI mnlI
haeIII/palI
hinlI/acyI
fnuDII/mvnI
mboII
sau96I
nlaIII
ahaII/bsaHI
bstUI
bpuAI
asuI
tru9I rcaI
aatII
bsh1236I
bbsI
eco0109I/draII
mseI bspHI
ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT

FIG. 3O

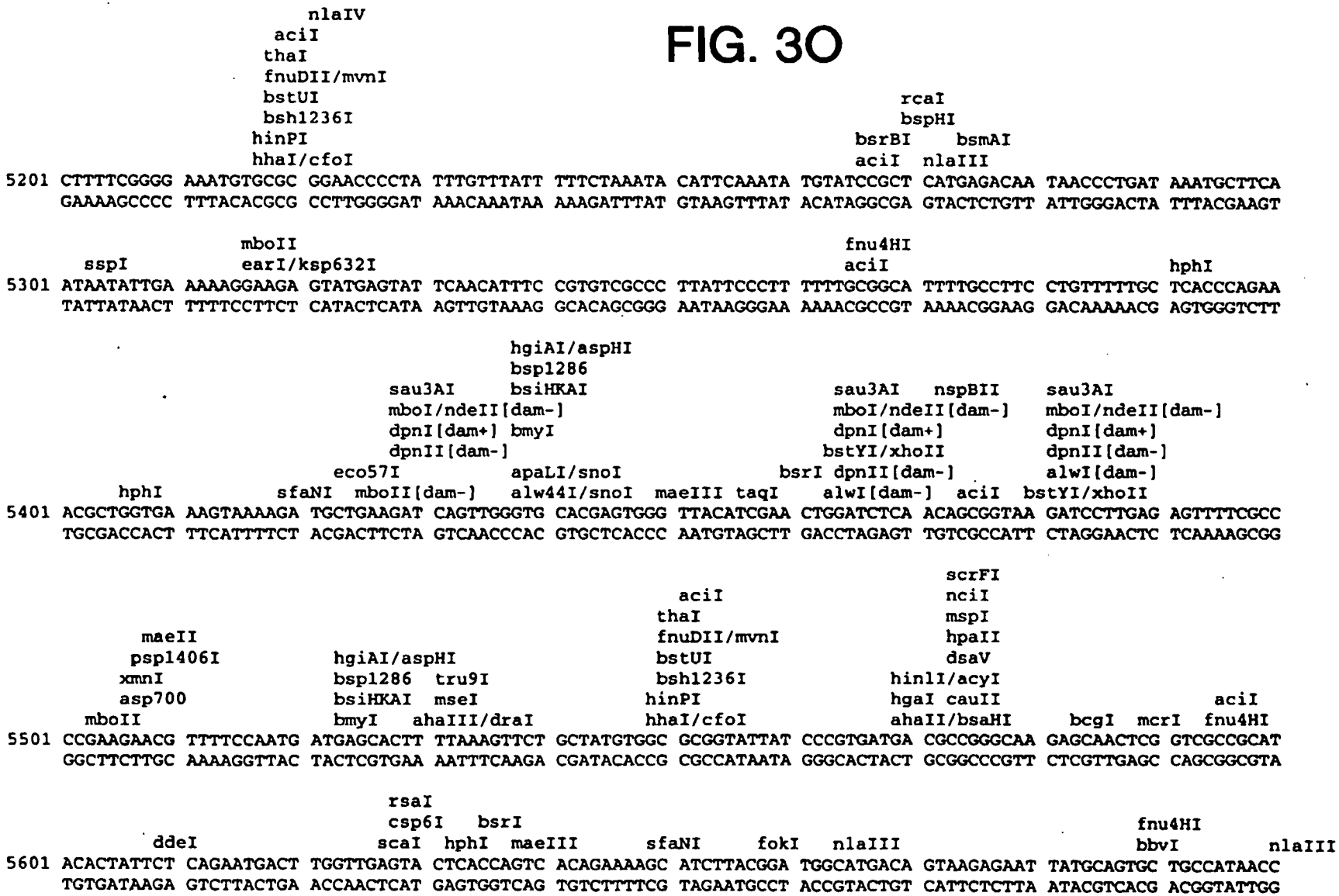

nlaIV
aciI
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
rcaI
bspHI
bsrBI bsmAI
aciI nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT
mboII
earI/ksp632I
fnu4HI
aciI
sspI
hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT
hgiAI/aspHI
bsp1286
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
bsiHKAI
bmyI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
bstYI/xhoII
nspBII
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
eco57I
apaLI/snoI
bsrI dpnII[dam-]
hphI
sfaNI mboII[dam-]
alw44I/snoI maeIII taqI
alwI[dam-] aciI bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG
scrFI
nciI
aciI
thaI
mspI
fnuDII/mvnI
hpaII
maeII
psp1406I
hgiAI/aspHI
bstUI
dsaV
xmnI
bsp1286 tru9I
bsh1236I
hinII/acyI
asp700
bsiHKAI mseI
hinPI
hgaI cauII
aciI
mboII
bmyI ahaIII/draI
hhaI/cfoI
ahaII/bsaHI
bcgI mcrI fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA
rsaI
csp6I bsrI
fnu4HI
ddeI
scaI hphI maeIII
sfaNI
fokI nlaIII
bbvI
nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG

FIG. 3P

```
                                                sau96I
                                                avaII
                                          sau3AI asuI                                                    nlaIII
                    haeIII/palI           mboI/ndeII[dam-]                                            sau3AI maeIII
                   eaeI                   dpnI[dam+]                                                  mboI/ndeII[dam-]
                   cfrI                   dpnII[dam-]                                                 dpnI[dam+]
                  fnu4HI                 pvuI/bspCI                                                   dpnII[dam-]
                  aciI                   mcrI    mnlI          aluI    aciI                    nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC
     TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG

                                                                                                   hinPI
                mspI                                                                               hhaI/cfoI
     sau3AI   nlaIV                                                                               mstI
     mboI/ndeII[dam-] aluI                                                                        aviII/fspI     bsrI
     dpnI[dam+]   hpaII                                                        fnu4HI      maeII           tru9I
     dpnII[dam-] bsaWI                                          maeIII     sfaNI  bbvI      psp1406I          mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
     AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG

                          mspI
                          hpaII                                                                     bglI
                         scrFI                                                                     sau96I
                    aluI  nciI        tru9I     fokI                          sau96I               haeIII/palI
                   rmaI   dsaV        mseI    bsrI        aciI                avaII        hinPI   asuI     mspI
                   maeI   cauII      aseI/asnI/vspI  mnlI                     asuI         hhaI/cfoI        hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
     ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG

                           mspI             thaI
                           hpaII            fnuDII/mvnI            haeIII/palI
                          cfr10I            bstUI                 sau96I
                        nlaIV hphI      bsmAI aciI         fnu4HI  nlaIV
                     gsuI/bpmI         bsaI  bsh1236I      bbvI bsrI asuI              mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
     ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

                                                    ddeI
                                                sau3AI      nlaIV
               pleI                             mboI/ndeII[dam-]
               hinfI                            dpnI[dam+]  hgiCI       tru9I
          eam1105I           fokI               dpnII[dam-] banI mnlI   mseI       maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
     TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT
```

FIG. 3Q

```
                                                    rmaI        sau3AI
                                                sau3AI hphI    mboI/ndeII[dam-]
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]     dpnI[dam+]
                                                dpnII[dam-]    dpnII[dam-]
                          tru9I             tru9I bstYI/xhoII  alwI[dam-]         nlaIII             maeII
                          mseI        tru9I mseI  alwI[dam-]  bstYI/xhoII         rcaI             tru9I
                         ahaIII/draI  mseI ahaIII/draI maeI  mboII[dam-]          bspHI            mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                sau3AI
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]   sau3AI
                                                dpnII[dam-]  mboI/ndeII[dam-] thaI
                                               bstYI/xhoII   dpnI[dam+]       fnuDII/mvnI
                                         sau3AI alwI[dam-]   dpnII[dam-]      bstUI
                                         mboI/ndeII[dam-]    alwI[dam-]       bsh1236I
                    hgaI                 dpnI[dam+] mboII[dam-]              hinPI       fnu4HI
                 ddeI                    dpnII[dam-]       bstYI/xhoII       hhaI/cfoI   bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                  sau3AI
                                  mboI/ndeII[dam-]
                                  dpnI[dam+]
                                  dpnII[dam-]
                                 alwI[dam-]
                  aciI          mspI                              bsrI                  hinPI
           aciI  nspBII         hpaII     aluI                  maeIII    eco57I        hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                   fnu4HI
                                                                                              alwNI    bbvI
                                                                                             bsrI  fnu4HI
       rmaI                haeIII/palI                                                                             
       maeI       bslI    haeI                 scfI    aciI         mnlI                maeIII    bbvI    bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R


scrFI
nciI
mspI
hpaII
dsaV
cauII
pleI
hinfI
mspI
hpaII
bsaWI
maeIII
aciI
nspBII
fnu4HI
bbvI
hinPI
mcrI
hhaI/cfoI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI
apaLI/snoI
alw44I/snoI
aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA
mspI
hpaII
bslI
fnu4HI
hinPI
hhaI/cfoI
ddeI
scfI
haeII
aciI
bsaWI
aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC
scrFI
mvaI
ecoRII
dsaV
bstNI
bsaJI
apyI[dcm+]
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
hinPI mnlI
hhaI/cfoI
aluI
taqI
mnlI drdI
hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA
haeIII/palI
haeIII/palI
fnu4HI
aciI
thaI bslI
fnuDII/mvnI
bstUI
bsh1236I
scrFI
mvaI bslI
ecoRII
dsaV
bstNI
apyI[dcm+]
nlaIII
nspI
haeIII/palI
nspHI
aflIII
sfaNI
nlaIV
aciI
nlaIV haeI
haeI
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT
fnu4HI
bbvI
fnu4HI
bbvI pleI
tfiI
hinfI
aciI
aluI
bsrBI
aciI
fnu4HI
aciI
mcrI
hinPI hinfI
hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT

FIG. 3S

```
                                                          thaI
                                                          fnuDII/mvnI
                                                          bstUI
                                                          bsh1236I
                                                         hinPI
                                                         hhaI/cfoI
                                                        thaI
                                                        fnuDII/mvnI
                 sapI  hinPI                            bstUI   haeIII/palI        aluI
                 mboII hhaI/cfoI                        bsh1236I          tru9I  pvuII
                 earI/ksp632I                mnlI       bslI    eaeI  tfiI aseI/asnI/vspI
           mnlI  aciI    haeII            aciI          aciI    cfrI  hinfI mseI   nspBII          bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
                             tru9I                          nlaIV bstNI
                     hinPI   mseI      maeIII               hgiCI apyI[dcm+]             mspI
         aciI        hhaI/cfoI aseI/asnI/vspI  mnlI         banI bsaJI                   hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                               tru9I
                                                               mseI
                                                              aseI/asnI/vspI
            aciI                                            xmnI
          bsrBI                       aluI      nlaIII      asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT

>length: 7360
```

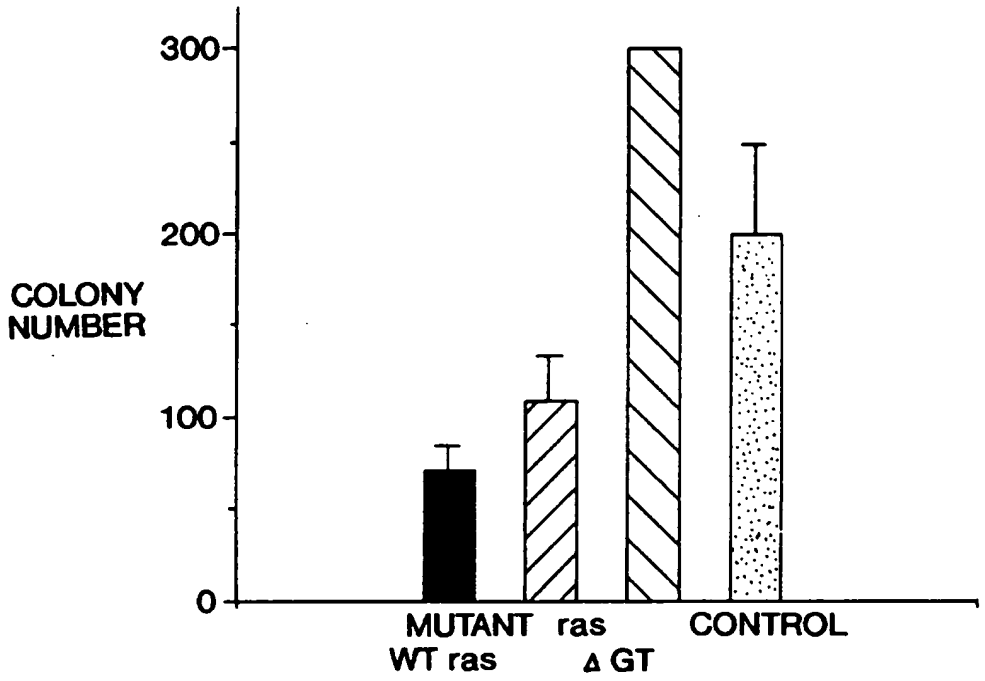

300
200
100
0
COLONY NUMBER
MUTANT ras
WT ras
Δ GT
CONTROL

FIG. 4

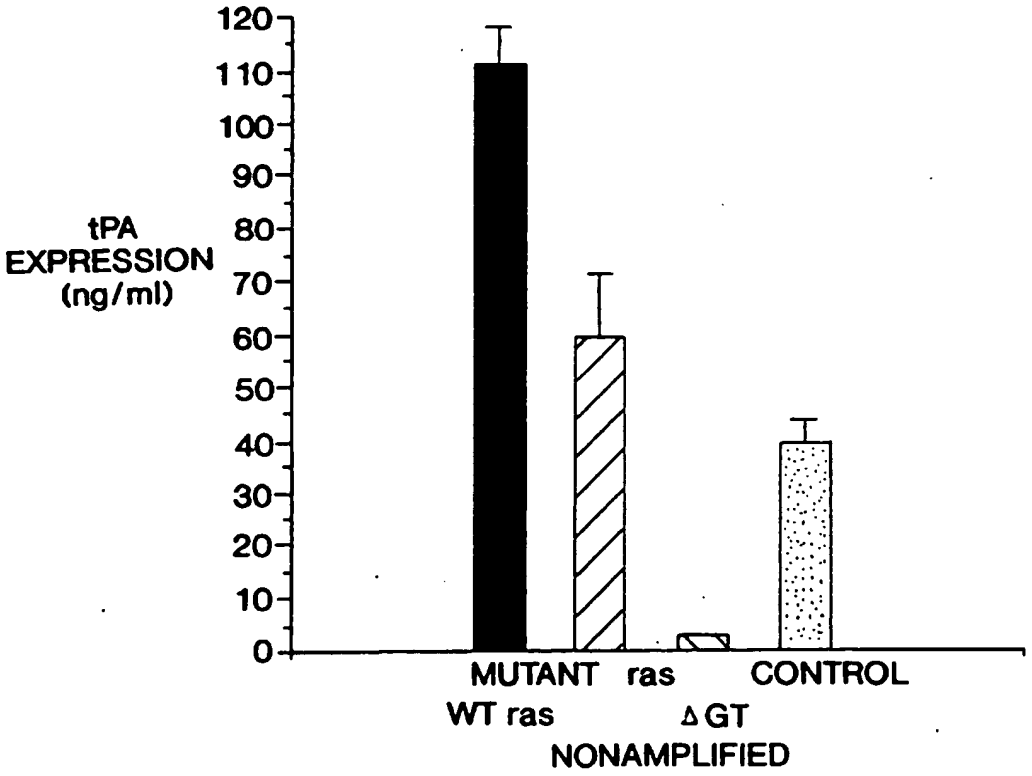

120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
MUTANT ras
WT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

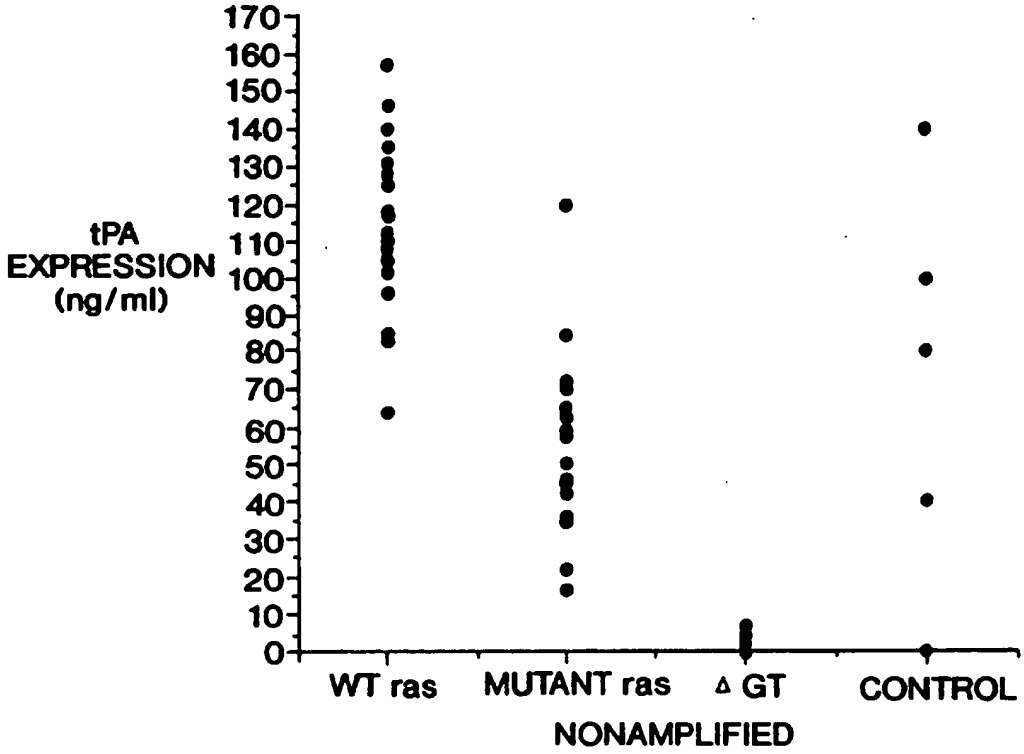

tPA
EXPRESSION
(ng/ml)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

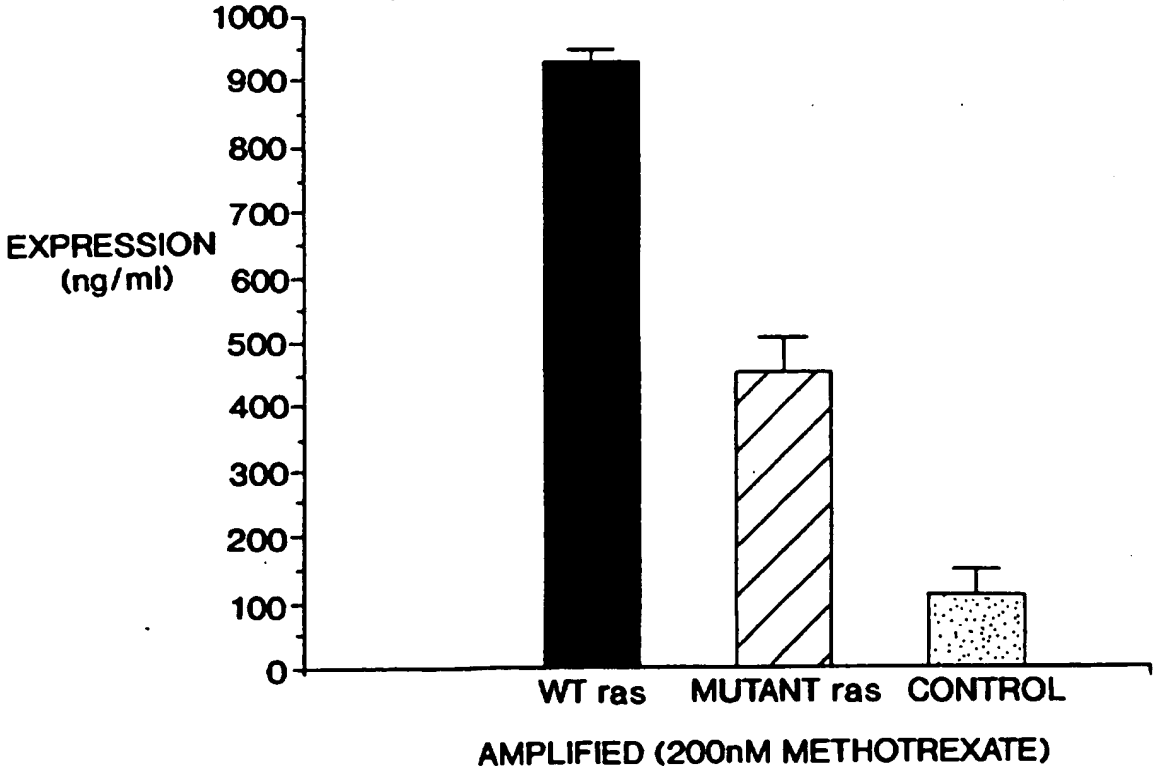

EXPRESSION
(ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 6A

```
      aluI
      sstI
      sacI
      hgiJII
      hgiAI/aspHI
      ecl136II
      bsp1286                                                                                    thaI
      bsiHKAI                                                                                    fnuDII/mvnI
      bmyI                          rmaI    tru9I                                                bstUI
      banII                         maeI    mseI                                                 bsh1236I
    taqI                            speI    aseI/asnI/vspI                        bslI           aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                              maeII
                sau96I                                   hinlI/acyI
                haeIII/palI     aciI                     ahaII/bsaHI
                asuI  apyI[dcm+]                aciI     aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                  maeII
     hinlI/acyI                                                                              hinlI/acyI
     ahaII/bsaHI                                    rsaI                    rsaI            ahaII/bsaHI
     aatII                                bglI      csp6I          ndeI     csp6I           aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
        aciI                                                                                 nlaIII
      bglI  dsaV                                                                      styI
     sau96I bstNI            rsaI                                        maeII              ncoI
     haeIII/palI             csp6I                                rsaI   snaBI              dsaI hphI  aciI
     asuI   apyI[dcm+]    bsrI  nlaIII                             csp6I  bsaAI              bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

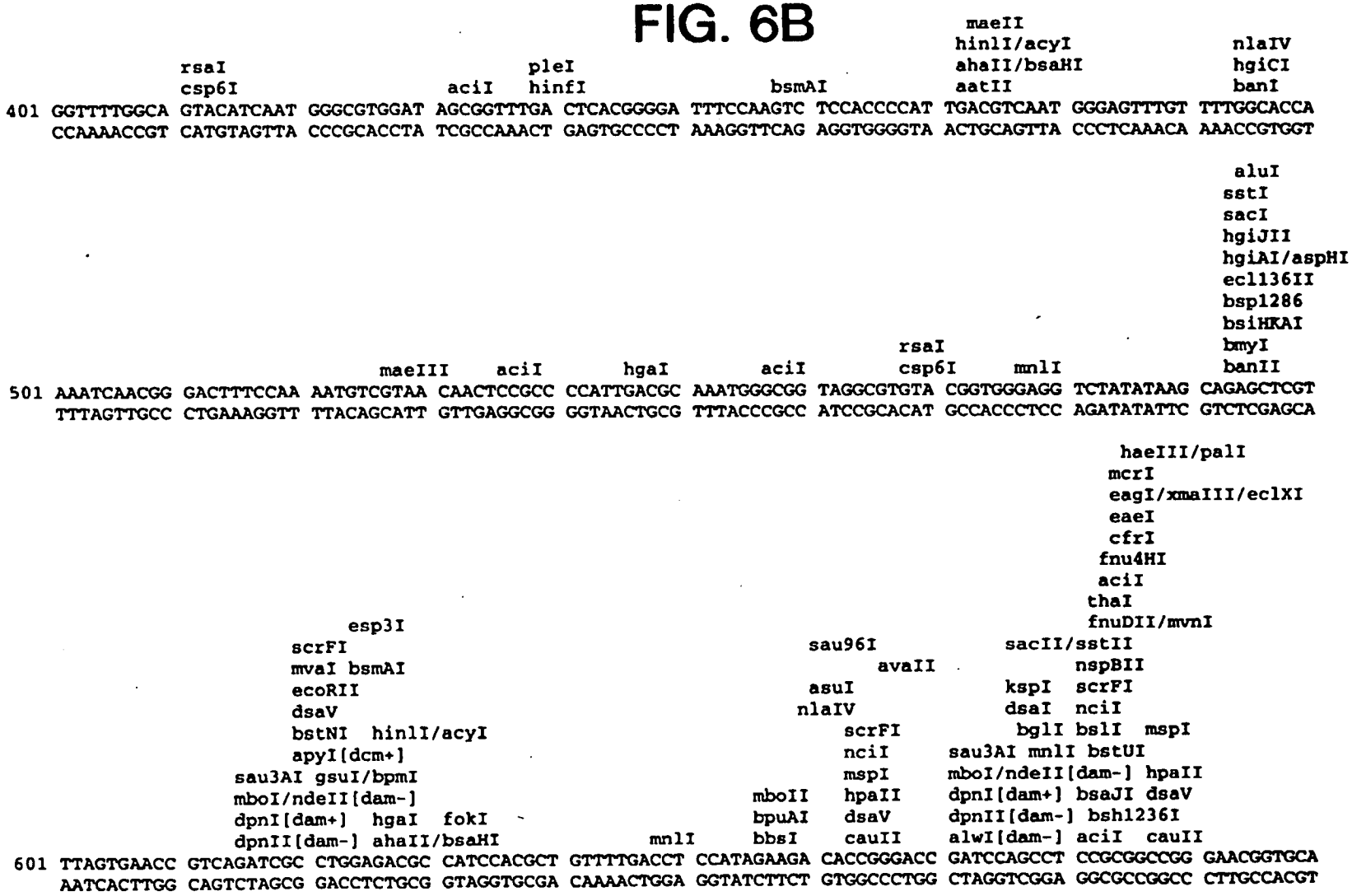

maeII
hinlI/acyI
ahaII/bsaHI
aatII
nlaIV
hgiCI
banI
rsaI
csp6I
pleI
aciI
hinfI
bsmAI
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
bmyI
banII
rsaI
csp6I
mnlI
maeIII
aciI
hgaI
aciI
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
fnu4HI
aciI
thaI
fnuDII/mvnI
esp3I
scrFI
mvaI bsmAI
ecoRII
dsaV
bstNI hinlI/acyI
apyI[dcm+]
sau3AI gsuI/bpmI
mboI/ndeII[dam-]
dpnI[dam+] hgaI fokI
dpnII[dam-] ahaII/bsaHI
sau96I
avaII
asuI
nlaIV
scrFI
nciI
mspI
mboII hpaII
bpuAI dsaV
mnlI bbsI cauII
sacII/sstII
nspBII
kspI scrFI
dsaI nciI
bglI bslI mspI
sau3AI mnlI bstUI
mboI/ndeII[dam-] hpaII
dpnI[dam+] bsaJI dsaV
dpnII[dam-] bsh1236I
alwI[dam-] aciI cauII
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT

FIG. 6C

```
                tfiI
             aciI
            thaI hinfI                                                                  fnu4HI
            fnuDII/mvnI                          aciI                                   bbvI
            bstUI                              rsaI                                   nspBII          taqI
            bsh1236I                           csp6I   scfI          mnlI             aciI       nlaIII
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                        thaI
                                                                                        fnuDII/mvnI
                                                                                        bstUI
                                                                                        bsh1236I
                                                                                      mluI
                                                                          bsrBI       aflIII      rsaI
                                                                          aciI      xmnI          csp6I
                    pflMI                          bsmAI                  mnlI   ddeI asp700      scaI
    sfaNI           bslI                           bsaI
801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
    CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
       eco57I                                                     dsaV
      mboII                    tfiI                             bstNI                    tfiI       tru9I          tru9I
     earI/ksp632I              hinfI                            apyI(dcm+)          mboII  taqI    mseI           mseI
    mnlI                     alwNI    hphI                    sexAI              ddeI    hinfI     ahaIII/draI    aseI/asnI/vspI
901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
    TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                          aluI
                                          sstI
                                          sacI
                                          hgiJII
                                          hgiAI/aspHI
                                          ecl136II
                                          bsp1286
                                          bsiHKAI
                                          bmyI                                          tru9I              mspI
                                          banII                                   sfaNI mseI               hpaII
          ddeI                    bslI    mnlI                  bstXI      fokI       aflII/bfrI          bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                        nlaIII
                                                       mvaI                 mvaI                            sau3AI
                                                       ecoRII         ecoRII                    mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI              dpnI[dam+]
                                                       bstNI     nlaIII     bstNI     ddeI                  dpnII[dam-]
             accI  nlaIII            mnlI              apyI[dcm+]   hinfI   apyI[dcm+]    hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI         scrFI
                                                                                     ahaII/bsaHI        mvaI
                                                                                  scrFI           ecoRII
                                                                                  mvaI    ecoNI         dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI      asuI    mnlI
                       aflIII                                                     apyI[dcm+]      mnlI  bstNI
         apoI       maeIII                                           mnlI           bsaJI hgaI      ddeI    apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                   ppu10I
                                                             sfaNI                                 nsiI/avaIII
         sfaNI                 accI     mboII            mboII                        mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                          styI               bstUI       mseI                                      maeIII
       bsaJI                         bsaJI              bsh1236I   aseI/asnI/vspI                            hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6E

```
                                                                                                       haeIII/palI
                                                                                                      eaeI
                                                           scrFI                                cfrI
                                                           mvaI                                      mspI
                                                           ecoRII                             hpaII
                                                           dsaV                               scrFI
                                                           bstNI                                    nciI
                                                           apyI[dcm+]      mnlI              ecoRI  dsaV
                                                         gsuI/bpmI         bsaJI      taqI  apoI   cauII
           scfI        fokI                           maeIII    hincII/hindII        claI/bsp106  bsaJI      aluI
    1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
         GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                             gsuI/bpmI
                                                            scrFI
                                                            mvaI
                                                            ecoRII                                          mnlI
                                                            dsaV                                            ecoNI
                  mnlI                           nspBII     bstNI                        ddeI                 sau96I
                haeIII/palI                  fnu4HI     hgiAI/aspHI                   mnlI                  nlaIV
               sau96I                     fnu4HI aciI   bsp1286                       eco81I                avaII
               asuI                       bbvI  fnu4HI  bsiHKAI    aluI               bsu36I/mstII/sauI     asuI  hphI
            nlaIII                   bspMI   bbvI       bmyI apyI[dcm+]             bslI                bsrI   bslI
    1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
         ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

            rmaI
            maeI
           styI
           bsaJI
           blnI            mboII                  styI                                         rsaI
           avrII           earI/ksp632I           bsaJI           mnlI         taqI        csp6I            nlaIV
    1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
         GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6F

```
                              scrFI
                              nciI
                              mspI
                              hpaII
                              dsaV
                              cauII
                             xmaI/pspAI
                             smaI
                             scrFI
                             nciI
                             dsaV
                             cauII
                             bslI
                           sau96I
                           haeIII/palI
                           asuI
                        scrFI
                        mvaI bsaJI
                        ecoRII
                        dsaV                                          nlaIV
        rsaI .          bstNI bsaJI           scfI                        fnu4HI                                alwNI
        csp6I           bslI avaI             pstI                        aciI          aciI               ddeI
       bsp1407I         apyI[dcm+]            bsgI                     bsrBI        hphI   eco57I          mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                              scrFI
                                                      mboII                   nciI
                                                     earI/ksp632I             mspI
       fnu4HI                                     sau3AI                      hpaII
      aluI                                         mboI/ndeII[dam-]           dsaV                   scfI              mspI
     pvuII                                         dpnI[dam+]                 cauII                  pstI              hpaII
     nspBII                                        dpnII[dam-]             sau96I                    bsgI           rsaI
   ddeI                                           bstYI/xhoII              avaII                    fnu4HI          csp6I
  mnlI bbvI                                       bglII                    asuI       draIII        bbvI    mboII  bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6G

FIG. 6H

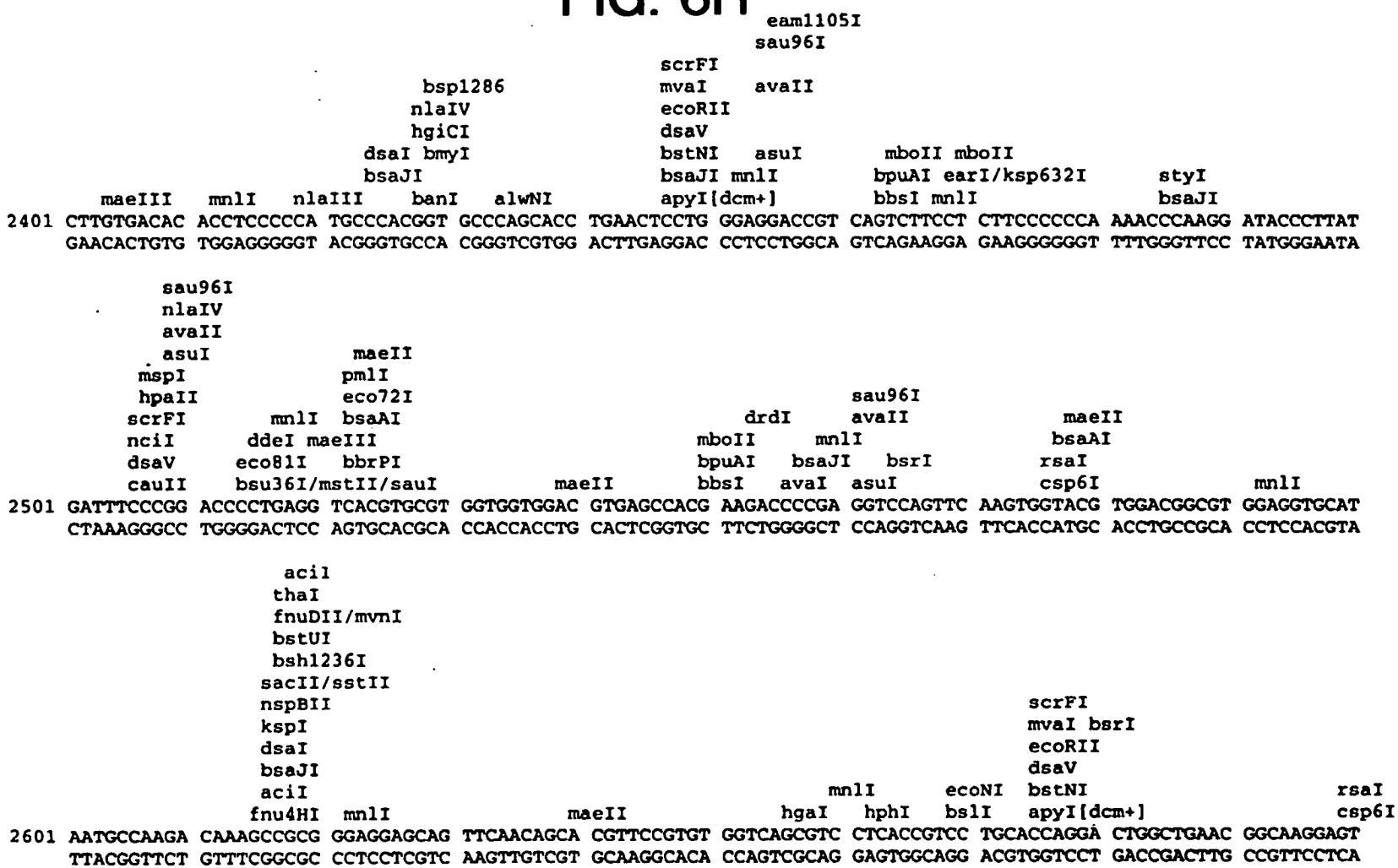


FIG. 6I

```
                bsmAI                                                                                    rsaI
                bsaI              mnlI              taqI                                 avaI           csp6I
                                                                                                         bsp1407I     bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

          scrFI
          nciI
          mspI
          hpaII
          dsaV
          cauII
          xmaI/pspAI
          smaI
          scrFI                    scrFI          scrFI
          nciI                     mvaI                mvaI
          dsaV                     ecoRII         ecoRII
          cauII                    dsaV           dsaV                                                aciI
          bsaJI                    bstNI                bstNI                                dsaI              nspBII
        fokI                       apyI[dcm+]           apyI[dcm+]                           bslI              fnu4HI fnu4HI
     bslI avaI  mnlI               sexAI           bspMI                                   bsaJI             bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                          dsaI
       mspI                                    pleI                    mnlI            hphI                    fnu4HI
       hpaII                          mnlI  nlaIII hinfI     nlaIV   mboII   scfI     aluI  bsaJI        bspMI   bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                                scrFI
                                                                                                                nciI
                                                                                                                mspI
                                                                                                                hpaII
                                                                                                                dsaV
                                                                                          scrFI                 cauII
                                                                                          nciI                  bsaJI
                                                                                          mspI                haeIII/palI
                                    nlaIII                                                hpaII               mcrI
                                  ppu10I                                  sapI            dsaV                eagI/xmaIII/eclXI
       xmnI mboII                 nsiI/avaIII                             mboII  mnlI      bsmAI               eaeI
       asp700      nlaIII     sfaNI   mnlI          aciI                 earI/ksp632I  bslI   cauII          cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6J

```
           rmaI
        mnlI
     sau3AI
     mboI/ndeII[dam-]                            styI
     dpnI[dam+]                               aciI
     dpnII[dam-]  taqI                       fnu4HI   sau96I
     alwI[dam-]  salI    scfI                sfiI ncoI haeIII/palI
    nlaIV  maeI  hincII/hindII               haeIII/palI
    bstYI/xhoII  accI    pstI               eaeI  dsaI asuI                    aluI
    bamHI xbaI pleI      bsgI    aluI cfrI  bsaJI                           fnu4HI
    alwI[dam-] hinfI bspMI      hindIII bglI nlaIII                         bbvI         maeIII          sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                                   sau3AI
                                                                                                   mboI/ndeII[dam-]
                                                                                                   dpnI[dam+]
                                                                                                   dpnII[dam-]
                                                                                                  pvuI/bspCI
                                                                                                  mcrI
                                                                                                taqI[dam-]    tru9I
                                                                                               claI/bsp106[dam-]
                                                                                              sau3AI         mseI
                                                                                              mboI/ndeII[dam-]
                                                                                              dpnI[dam+] xmnI        hinPI
                                           rmaI                                               dpnII[dam-]  aseI/asnI/vspI
                                   bsmI  maeI                                          nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                       rsaI
                                                        csp6I
           haeI                                        nlaIV
        styI                                           kpnI
        ncoI                                           hgiCI
        dsaI haeIII/palI                               banI                  aluI
     fnu4HI nlaIII                                     asp718     mnlI      pvuII
     bbvI  bsaJI          mnlI      mnlI               acc65I  ddeI aciI    nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6K

```
                nlaIV                                                                                                  nlaIV
              scrFI                                                                  scrFI        scrFI
              mvaI                                ppu10I                               mvaI                 mvaI
              ecoRII                            nsiI/avaIII                        ecoRII       ecoRII
              dsaV                              nlaIII                             dsaV         dsaV
              bstNI                                sphI                                 bstNI                bstNI
              apyI[dcm+]                           nspI sfaNI                           apyI[dcm+]           apyI[dcm+]
            bsaJI                                  nspHI                              sexAI              bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

                  sfaNI
                ppu10I
                nsiI/avaIII
               nlaIII
              sphI
              nspI                                                                  aciI                                                  bslI
              nspHI                                    aciI          aciI  fokI              aciI bsrI  aciI                          aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                                        rmaI
                                                                                                                       styI
                                                                                                                       bsaJI
                                                  fnu4HI                                                               blnI
       nlaIII                                     sfiI mnlI                                                            avrII
      styI                                        haeIII/palI                                                        haeIII/palI
      ncoI                                  bsaJI bglI          ddeI                                                 stuI
      dsaI                                 haeIII/palI bsaJI  mnlI  aluI                  mnlI                       haeI
      bsaJI                               mnlI  mnlI aciI    haeIII/palI                mnlI                       mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                                  scrFI
                                                                                  mvaI
                                                                                  ecoRII
                    tru9I             haeIII/palI                                 dsaV
                   hpaI               eaeI                                        bstNI
                   hincII/hindII      cfrI                        bsrI            apyI[dcm+]          tru9I        fnu4HI
                aluI mseI  aluI     bsrI                 maeII maeIII           bsaJI   maeIII      mseI         bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6L

```
                                                                                                                   hinPI
                                                                                                                  hhaI/cfoI
                                                                                                                  nlaIV
                                                            sau3AI                                                narI
                                                  sau96I    mboI/ndeII[dam-]                                      kasI
                                                  haeIII/palI                                                     hinlI/acyI
                                                  asuI      dpnI[dam+]                                            hgiCI
                          aluI                  mnlI        dpnII[dam-]                                           haeII      aciI
                         pvuII               mboII    aciI pvuI/bspCI                                             banI    sfaNI
          fokI           nspBII              earI/ksp632I  mcrI                                       bglI        ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                                      aciI
                                                                                                                   fnu4HI
                                                                                  hinPI                            aciI
                                                                                 thaI                             thaI
                                                                                 fnuDII/mvnI                      fnuDII/mvnI
                                                                                 bstUI scfI     hinPI             bstUI
                                                                                 bsh1236I       hhaI/cfoI hinPI
                                                                             rsaI hhaI/cfoI  fnu4HI  tru9I bsh1236I
                          sfaNI     aciI          aciI   maeII               csp6I    bslI    aciI    mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

           fnu4HI
         hinPI
         hhaI/cfoI                                     hinPI
        thaI                                           hhaI/cfoI
        fnuDII/mvnI                                   rmaI                                                      mspI
        bstUI                                    hinPI  haeII                                                   hpaII
        bsh1236I     aciI                        hhaI/cfoI    bsrBI                                            naeI
     maeIII bbvI  maeIII                         haeII maeI    aciI            mboII                    maeII  cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                             nlaIV
                           hgiJII
                           bsp1286                                nlaIV                                        maeII     haeIII/palI
                            bmyI                                  hgiCI  taqI                                 draIII    sau96I
      aluI                  banII          nlaIV                  banI mnlI                         hphI        bsaAI     asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6M

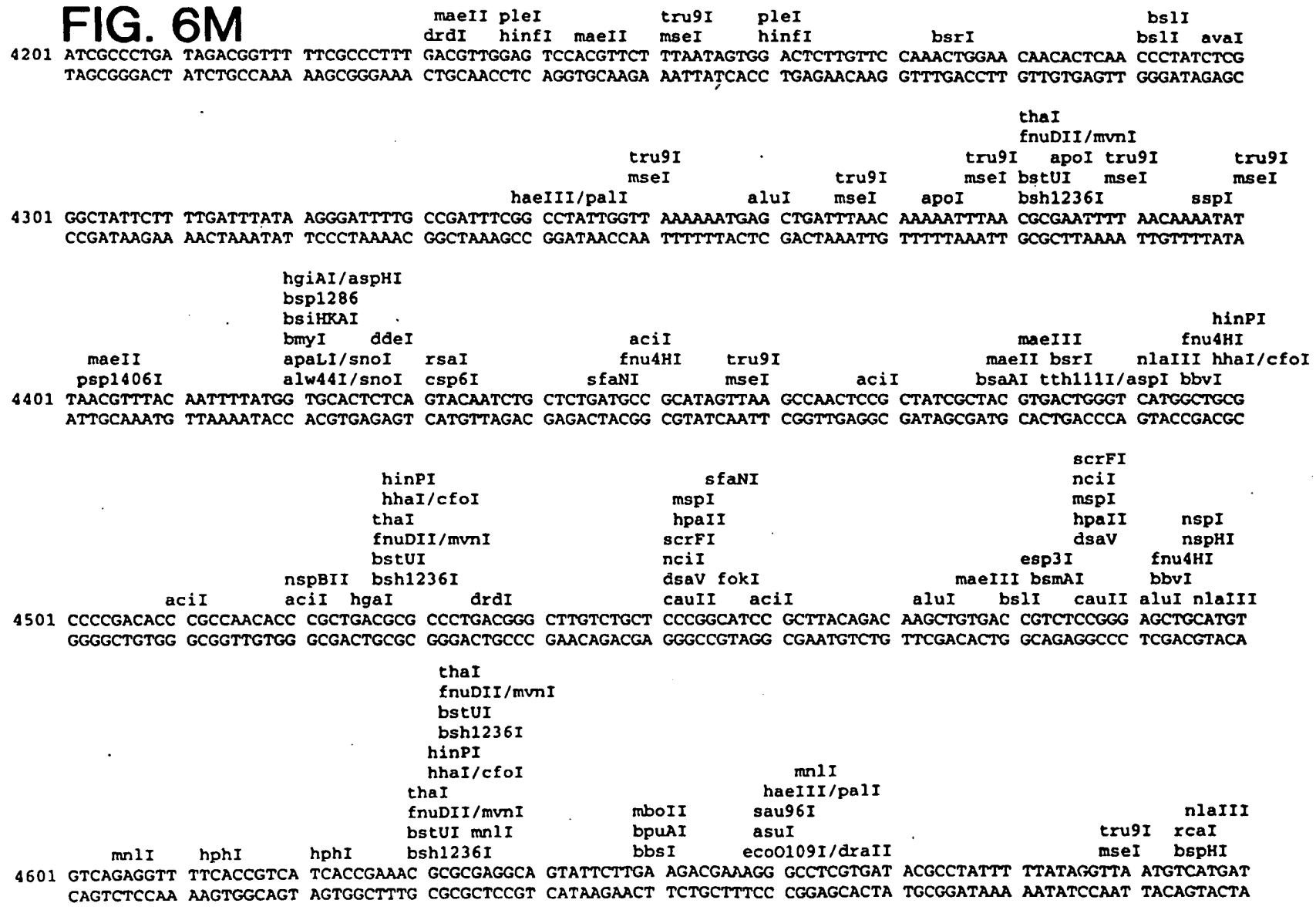


FIG. 6N

```
                                                          nlaIV
                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                     hinlI/acyI                        bstUI                                                    rcaI
                     ahaII/bsaHI                       bsh1236I                                                 bspHI
                     aatII                            hinPI                                                 bsrBI
                 ddeI maeII                           hhaI/cfoI                                             aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                     mboII                                                 fnu4HI
       bsmAI                           sspI          earI/ksp632I                                          aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                             hgiAI/aspHI
                                                                             bsp1286
                                                                  sau3AI     bsiHKAI                      sau3AI
                                                                  mboI/ndeII[dam-]                        mboI/ndeII[dam-]
                                                                  dpnI[dam+] bmyI                         dpnI[dam+]
                                                                  dpnII[dam-]                            bstYI/xhoII
                                                             eco57I          apaLI/snoI              bsrI dpnII[dam-]
                         hphI             hphI          sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI    alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                           aciI
              sau3AI                                                                      thaI
              mboI/ndeII[dam-]              maeII                                         fnuDII/mvnI
              dpnI[dam+]                   psp1406I          hgiAI/aspHI                  bstUI
              dpnII[dam-]                 xmnI               bsp1286 tru9I                bsh1236I             hinlI/acyI
       aciI   alwI[dam-]                  asp700             bsiHKAI mseI                hinPI                 hgaI
     nspBII  bstYI/xhoII               mboII                 bmyI   ahaIII/draI          hhaI/cfoI             ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

      scrFI
      nciI
      mspI
      hpaII                                                       rsaI
      dsaV                    aciI                                csp6I   bsrI
      cauII      bcgI  mcrI  fnu4HI          ddeI                scaI  hphI  maeIII      sfaNI     fokI   nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6O

```
                                                                                                                    sau96I
                                                                                                                    avaII
                                                                                                          sau3AI    asuI
                                                                                haeIII/palI                         mboI/ndeII[dam-]
                                                                               eaeI                                 dpnI[dam+]
                                                                               cfrI                                 dpnII[dam-]
                                                fnu4HI                        fnu4HI                              pvuI/bspCI
                                                bbvI          nlaIII          aciI                                mcrI    mnlI          aluI    aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
     ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA

                                  maeIII
                                 nlaIII
                               sau3AI                          mspI
                               mboI/ndeII[dam-]    sau3AI  nlaIV
                               dpnI[dam+]          mboI/ndeII[dam-] aluI
                               dpnII[dam-]         dpnI[dam+]   hpaII                                                                fnu4HI
                      nlaIII alwI[dam-]            dpnII[dam-] bsaWI                                          maeIII     sfaNI   bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
     CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT

                                                                    mspI
                                hinPI                               hpaII
                               mstI                                 scrFI
                               aviII/fspI     bsrI                  aluI  nciI        tru9I      fokI                    sau96I
                         maeII hhaI/cfoI  tru9I                     rmaI  dsaV        mseI    bsrI        aciI            avaII
                        psp1406I          mseI                      maeI  cauII     aseI/asnI/vspI   mnlI               asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
     TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG

                                bglI                                mspI              thaI
                               sau96I                               hpaII             fnuDII/mvnI              haeIII/palI
                               haeIII/palI                          cfr10I            bstUI                   sau96I
                         hinPI  asuI    mspI                      nlaIV hphI       bsmAI aciI        fnu4HI   nlaIV
                         hhaI/cfoI      hpaII                   gsuI/bpmI          bsaI  bsh1236I     bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
     GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

                                                                                                 ddeI
                                                                                          sau3AI        nlaIV
                                                    pleI                                  mboI/ndeII[dam-]  mnlI
                                                    hinfI                                 dpnI[dam+]    hgiCI          tru9I
                        mnlI                      eam1105I              fokI              dpnII[dam-]   banI           mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
     ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA
```

FIG. 6P

```
                                                                                                       rmaI      sau3AI
                                                                                                   sau3AI hphI  mboI/ndeII[dam-]
                                                                                                   mboI/ndeII[dam-]
                                                                                                   dpnI[dam+]   dpnI[dam+]
                                                                                                   dpnII[dam-]  dpnII[dam-]
                                                                    tru9I              ahaIII/draI  maeI        alwI[dam-]
                                                                    mseI           tru9I tru9I bstYI/xhoII  bstYI/xhoII
           maeIII                                                  ahaIII/draI     mseI  mseI  alwI[dam-] mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                                 sau3AI
                                                                                                 mboI/ndeII[dam-]
                                                                                                 dpnI[dam+]  sau3AI
                                                                                                 dpnII[dam-] mboI/ndeII[dam-]
                                                                                                bstYI/xhoII  dpnI[dam+]
                                                                                        sau3AI  alwI[dam-]   dpnII[dam-]
        nlaIII              maeII                                                       mboI/ndeII[dam-]     alwI[dam-]
       rcaI              tru9I                         hgaI                             dpnI[dam+] mboII[dam-]
       bspHI             mseI                       ddeI                                dpnII[dam-]         bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                              sau3AI
      thaI                                                                    mboI/ndeII[dam-]
      fnuDII/mvnI                                                             dpnI[dam+]
      bstUI                                                                   dpnII[dam-]
      bsh1236I                                                               alwI[dam-]
     hinPI      fnu4HI                                 aciI            mspI                                   bsrI
     hhaI/cfoI  bbvI                         aciI    nspBII            hpaII      aluI                      maeIII   eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

              hinPI                        rmaI                   haeIII/palI
              hhaI/cfoI                    maeI      bslI        haeI                   scfI    aciI        mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6Q

```
                                                           scrFI                                              aciI
                                                           nciI                                             nspBII
                            fnu4HI                         mspI                       mspI              fnu4HI
                    alwNI   bbvI                           hpaII                      hpaII             bbvI
                  bsrI  fnu4HI                             dsaV       pleI           bsaWI            hinPI mcrI
            maeIII      bbvI    bsrI                       cauII      hinfI        maeIII             hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

                  hgiAI/aspHI
                  bsp1286
                  bsiHKAI
                  bmyI                                                                                  hinPI
                  apaLI/snoI                                                                            hhaI/cfoI
                  alw44I/snoI  aluI                                  ddeI      scfI                    haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                             scrFI
                                                                             mvaI          scrFI
                                                                             ecoRII  mvaI
                                 mspI                                        dsaV    ecoRII
                                 hpaII                                       bstNI         dsaV
                                 bslI    fnu4HI                   hinPI mnlI bsaJI         bstNI
                aciI            bsaWI    aciI                     hhaI/cfoI  aluI apyI[dcm+]  apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                      haeIII/palI
                                                                                                     fnu4HI
                                                                                                     aciI
                                                                                                    thaI bslI
                                                                                                    fnuDII/mvnI
                                       taqI                              nlaIV                      bstUI
                      mnlI drdI     hgaI          sfaNI               aciI                          bsh1236I   nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6R

```
      haeIII/palI
      haeI
     scrFI
     mvaI bslI
     ecoRII                nlaIII
     dsaV                 nspI
     bstNI          haeIII/palI  nspHI                    tfiI                                                    bsrBI
     apyI[dcm+]     haeI         aflIII                   hinfI                    aciI          aluI     aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                                  thaI
                                                                                                  fnuDII/mvnI
                                                                                                  bstUI
                                                                                                  bsh1236I
                                                                                                 hinPI
                                                                                                  hhaI/cfoI
                                                                                                 thaI
                                                                                                 fnuDII/mvnI
        fnu4HI              fnu4HI                    sapI  hinPI                               bstUI   haeIII/palI
        bbvI                bbvI pleI                 mboII hhaI/cfoI                           bsh1236I          tru9I
      aciI                 hinPI  hinfI               earI/ksp632I                 mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
     fnu4HI       mcrI     hhaI/cfoI          mnlI   aciI    haeII           aciI       aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                                  scrFI
                                                                                                  mvaI
                                                                                                  ecoRII
                                                                                                  dsaV
         aluI                                                  tru9I                            nlaIV bstNI
         pvuII                                          hinPI       mseI      maeIII             hgiCI apyI[dcm+]
         nspBII                       bsrI     aciI       hhaI/cfoI  aseI/asnI/vspI  mnlI       banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                                  tru9I
                                                                                                  mseI
                                                                                                 aseI/asnI/vspI
                mspI                          aciI                                              xmnI
                hpaII                        bsrBI                          aluI      nlaIII    asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT

>length: 6889
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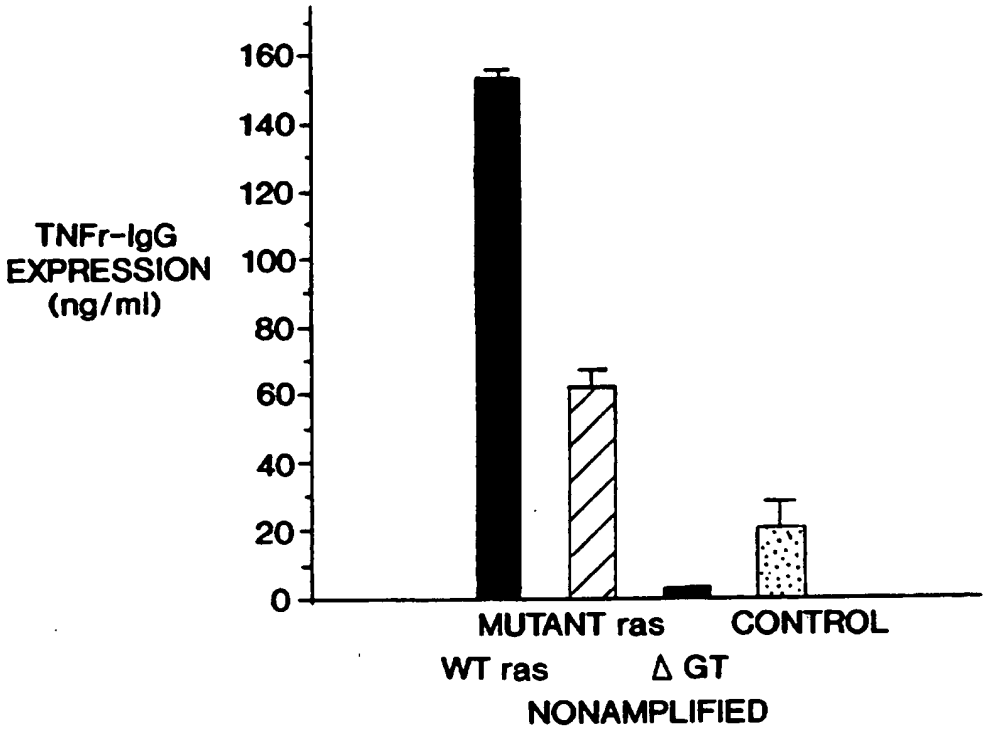
TNFr-IgG
EXPRESSION
(ng/ml)
160
140
120
100
80
60
40
20
0
MUTANT ras
CONTROL
WT ras
Δ GT
NONAMPLIFIED

FIG. 7A

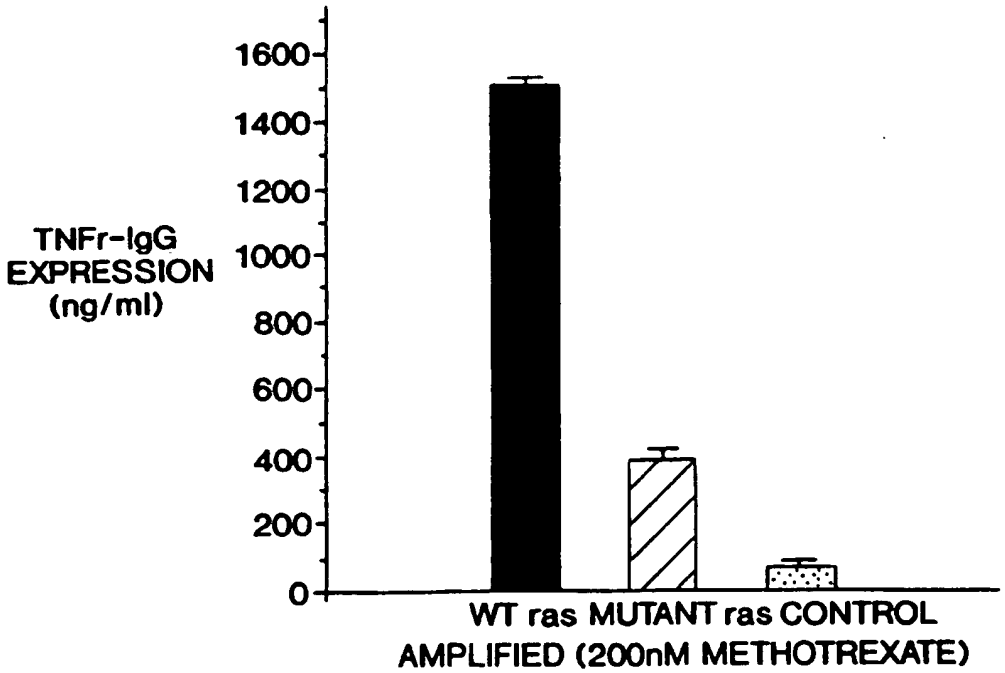
TNFr-IgG
EXPRESSION
(ng/ml)
1600
1400
1200
1000
800
600
400
200
0
WT ras MUTANT ras CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 8

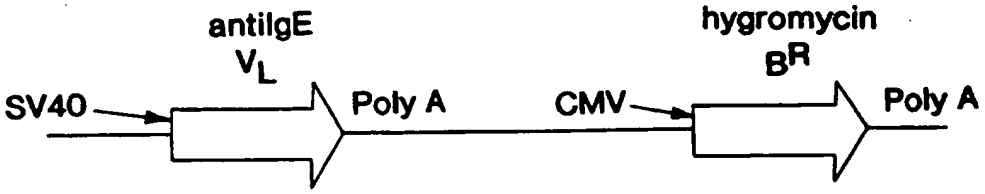
antiIgE
V_L
hygromycin
B^R
SV40
Poly A
CMV
Poly A

FIG. 11

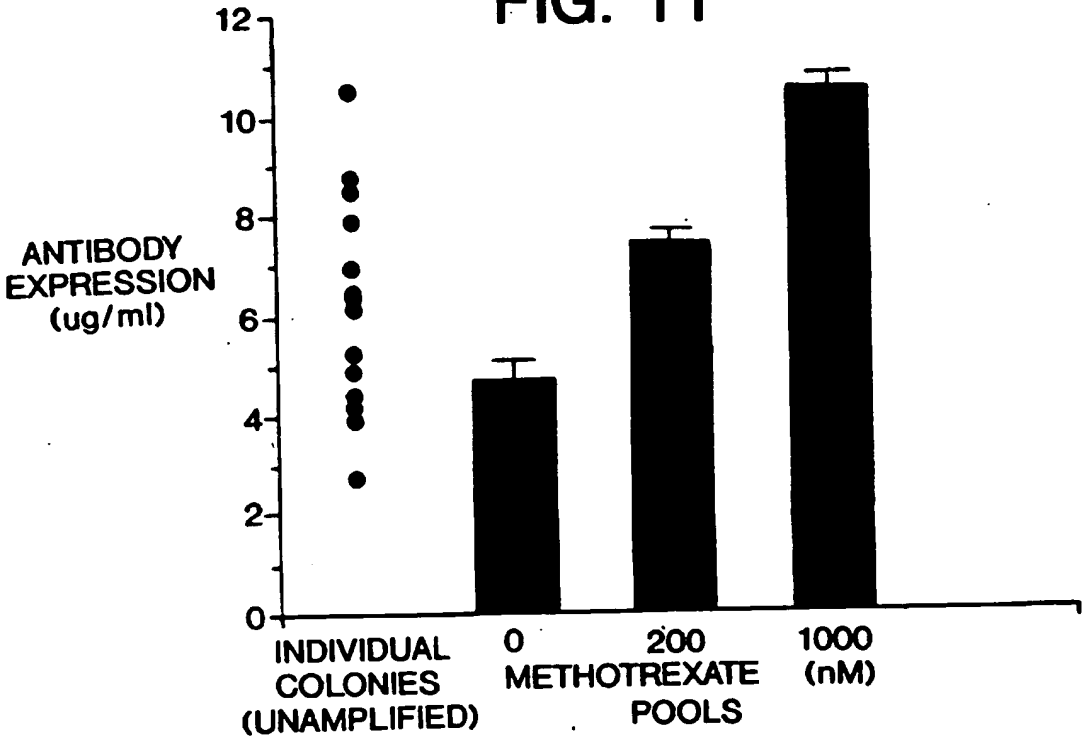
ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
METHOTREXATE (nM)
POOLS

FIG. 9A

```
      aluI
      sstI
      sacI                                                aluI
      hgiJII                                          sau3AI pvuII                                                                  nlaIV
      hgiAI/aspHI                                     mboI/ndeII[dam-]                                                            scrFI
      ecl136II                                        dpnI[dam+]                                                                  mvaI
      bsp1286                                        pvuI/bspCI                                                                   ecoRII
      bsiHKAI                                     pleI dpnII[dam-]                                                                dsaV
      bmyI                                        hinfI  taqI[dam-]                                                               bstNI
      banII                                    rmaI    mcrI    nspBII                                                             apyI[dcm+]
    taqI                                       maeI   taqI[dam-]                                                                bsaJI
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                                  nlaIV
                    sfaNI                  scrFI        scrFI                                                  sfaNI
                  ppu10I                   mvaI              mvaI                                                    ppu10I
                  nsiI/avaIII              ecoRII       ecoRII                                                 nsiI/avaIII
                 nlaIII                    dsaV         dsaV                                                  nlaIII
                sphI                       bstNI             bstNI                                                    sphI
                nspI                       apyI[dcm+]        apyI[dcm+]                                               nspI
                nspHI                      sexAI             bsaJI                                                    nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                                                  nlaIII
                                                                                                                 styI
                                                                                                                 ncoI
                                                  aciI                                                        bslI dsaI
                    aciI          aciI  fokI                 aciI bsrI  aciI                                  aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                                                  rmaI
                                                                                                 styI
                                                                                                 bsaJI                                haeIII/palI
                      fnu4HI                                                                     blnI                                mcrI
                      bglI                                                                       avrII                        aluI    eagI/xmaIII/eclXI
                     sfiI                                                                     haeIII/palI                    rmaI    eaeI
                     haeIII/palI                                                             stuI                           maeI    cfrI
                   mnlI   mnlI      ddeI                                                     haeI                 mnlI      nheI    mspI
          haeIII/palI bsaJI  mnlI   aluI                                                  mnlI maeI               aluI      hpaII
        mnlI bsaJI aciI    haeIII/palI                                                 mnlI
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 9B

```
                                tfiI
    scrFI                       hinfI
    nciI                    aciI
    mspI                    thaI                                                fnu4HI
    hpaII                   fnuDII/mvnI                 aciI                    bbvI
    dsaV                    bstUI                   maeII  rsaI                 nspBII
    cauII                   bsh1236I               maeIII    csp6I  scfI          mnlI          aciI        nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                    haeIII/palI
                                                                  haeI
                                                                scrFI
                                                                mvaI      bsrBI
                                                                ecoRII
                                                                dsaV
                                                                bstNI     aciI                      rsaI
                                    pflMI                       bsmAI     apyI[dcm+]          xmnI  csp6I
    taqI    .       sfaNI           bslI                        bsaI      bsaJI  mnlI  ddeI  asp700    scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                scrFI
                                                                mvaI
                                                                ecoRII
                        eco57I                                  dsaV
                       mboII                tfiI                bstNI                     tfiI       tru9I
                      earI/ksp632I          hinfI               apyI[dcm+]                hinfI      mseI
                     mnlI                 alwNI    hphI        sexAI              ddeI mboII taqI   ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                    sstI
                                                    sacI
                                                    hgiJII
                                                    hgiAI/aspHI
                                                    ecl136II
                                                    bsp1286
                                                    bsiHKAI
          tru9I                                     bmyI                                tru9I
          mseI          ddeI                        banII                               aflII/bfrI
         aseI/asnI/vspI                         bslI   mnlI aluI          bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

```
                                                                                                      haeIII/palI
                                                                                                     haeI
                                                                      scrFI          scrFI
                                                                      mvaI                 mvaI
                                                                      ecoRII         ecoRII
        mspI                                                          dsaV   tfiI    dsaV
        hpaII                                                         bstNI    nlaIII      bstNI    ddeI pleI
       bsaWI                  accI nlaIII           mnlI              apyI[dcm+]   hinfI   apyI[dcm+]    hinfI
 801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
     TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                    hgaI
                                                                                                   hinlI/acyI
                                                                                                   ahaII/bsaHI
                                                                                                scrFI
            nlaIII                                                                              mvaI     mnlI
        sau3AI                                                                                  ecoRII
        mboI/ndeII[dam-]                                                                        dsaV
        dpnI[dam+]                 maeII                                                        bstNI    ecoNI
        dpnII[dam-]               aflIII                                                        apyI[dcm+]    mnlI
     maeIII alwI[dam-]  apoI    maeIII                                          mnlI           bsaJI     bslI ddeI
 901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
     CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

        scrFI
        mvaI
        ecoRII
        dsaV
        bstNI
        apyI[dcm+]
      sau96I
      avaII                                                            sfaNI
      asuI   mnlI      sfaNI             accI     mboII                  mboII                     mnlI     aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                   styI
                                                   bsaJI
                                              sau3AI
                 nlaIII                       mboI/ndeII[dam-]
                styI                          dpnI[dam+]
                ncoI                          dpnII[dam-]          aluI     tru9I
     ppu10I     dsaI                          alwI[dam-]          fnu4HI    mseI
     nsiI/avaIII  bsaJI                      bstYI/xhoII          bbvI     aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9D

```
                                                                            sau96I
                                                                            avaII
                                                                            asuI
                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                                                                          dsaV
                                                                          bstNI                             ecoRI
                 maeIII                                                   apyI[dcm+]     mnlI        taqI   apoI
                hphI   scfI       fokI                             bslI  bsaJI           bsaJI      claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                         scrFI
         nlaIII                                                                                          mvaI
        styI                                                                                             ecoRII
        pflMI                                                                                            dsaV
        ncoI                                                                                             bstNI fnu4HI
        dsaI                                                   rsaI            aluI                      apyI[dcm+]
        bslI fokI                      rmaI             gsuI/bpmI             pvuII    pleI         haeI      bbvI
        bsaJI       nlaIII  fokI       maeI            bsrI   csp6I           nspBII   hinfI    aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9E

```
                                                                                                                   scrFI
                                                                                                                   nciI
                                                                                                                   mspI
                                                                                                                   hpaII
                                                                                                                   dsaV
                                                                                                                   cauII
                                                                                                                   bslI
                                                                                                                  xmaI/pspAI
                                                                                                                  smaI
                                                                                                                  scrFI
                                                                                                                  nciI
                                                                                                                  dsaV
                                                                                                                  cauII      scrFI
       hgiJII                                                                                                  sau96I       mvaI
       bsp1286                                                                                       sau3AI    nlaIV        ecoRII
       bmyI                                                                  mspI                    mboI/ndeII[dam-]       dsaV
  scrFI                                                                hpaII                   dpnI[dam+]    bslI      bstNI
  mvaI banII                                                                 mroI                    dpnII[dam-]  bsaJI       bslI
  ecoRII                                                             bspMII                  nlaIV       haeIII/palI     apyI[dcm+]
  dsaV                                                               bspEI                   bstYI/xhoII    avaI       sau96I
  bstNI                                                                    bsaWI                   bamHI     eco0109I/draII haeIII/palI
  bsaJI                                                                  mnlI                      alwI[dam-] asuI             asuI
  apyI[dcm+]                           alwNI                         hphI  accIII     aluI   bsrI alwI[dam-]  bsaJI     eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                                      sau3AI
                                      mboI/ndeII[dam-]
                                      dpnI[dam+]                                                           thaI
                            maeII     dpnII[dam-]                                                          fnuDII/mvnI
                      taqI snaBI     alwI[dam-]               hgaI      haeIII/palI                        bstUI   tfiI
                     claI/bsp106     mspI                   rmaI       sau96I                              bsh1236I
                 sfaNI     bsaAI     hpaII taqI[dam-]       maeI       asuI                               nruI    hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9F

```
                                                                                          scrFI
                                                                                          mvaI
                                                                                          ecoRII
                                                                                          dsaV
                                                                                          bstNI
                                                                                          apyI[dcm+]
                                                                                       hinPI
                                                                                       hhaI/cfoI
                                                         mnlI                         nlaIV
                                                      xhoI                            narI
                                                      paeR7I                          kasI
                                                      avaI                            hinlI/acyI
      scfI                                        hgiAI/aspHI                         hgiCI
      pstI                                        bsp1286                             haeII
      bsgI                     mnlI               bsiHKAI    fnu4HI                   banI
    bspMI                    ddeI drdI            bmyI taqI bbvI                      ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                         sau96I
                                         haeIII/palI
                                        sau96I
                                        nlaIV                                                               scrFI
                                        hgiJII                  nlaIV                                       mvaI
                                        bsp1286                 hgiCI                                       ecoRII
         scrFI                     bsp120I               banI                                               dsaV
         mvaI                          bmyI                 scrFI                                     bstNI
         ecoRII                    banII              mvaI                                            bsaJI
         dsaV                      asuI               ecoRII                                        sau96I
         bstNI hphI                    apaI        mboII   dsaV          hgiAI/aspHI             haeIII/palI
         apyI[dcm+] bsmAI     haeIII/palI eco0109I/draII   bstNI               bsp1286                asuI
       bsaJI maeIII     mnlI           styI asuI   bpuAI   apyI[dcm+]  mnlI    bsiHKAI           fnu4HI
    nlaIV   bstEII esp3I bsaJI mnlI    bsaJI       bbsI    bsaJI     mnlI      bmyI mnlI   bsp1286 aciI apyI[dcm+]
                                                                                           bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9G

```
          scrFI                                            hinPI
          mvaI                                                  nlaIV
          ecoRII                                          narI
          ecoNI                                                 kasI                hgiAI/aspHI
          dsaV                    hphI                    hinlI/acyI          bsp1286      mspI
          bstNI                       mspI                      hgiCI               bsiHKAI      hpaII
          bslI                        hpaII                     haeII               bmyI        scrFI
          apyI[dcm+]                cfr10I                      banI          fnu4HI            nciI
      fnu4HI                        bsaWI  tth111I/aspI         ahaII/bsaHI   aciI  apaLI/snoI  dsaV
      bbvI                    bslI  ageI maeIII             ddeI hhaI/cfoI  nspBII  alw44I/snoI cauII      scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                               nlaIV
      ddeI pleI                                       fnu4HI   hgiCI
     mnlI  hinfI                               mnlI   bbvI     banI                        tfiI
     eco81I      mnlI  fnu4HI  maeIII bsp1286 rmaI       bsp1286                      hinfI                styI
     bsu36I/mstII/sauI ddeI bbvI   hphI    bmyI    maeI aluI  bmyI                     maeII                bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                                eam1105I
                                                                                               sau96I
                                                                                       scrFI
                                                                                       mvaI    avaII
                                                                                       ecoRII
                         hgiJII                                                        dsaV
                         bsp1286                        nlaIII                         bstNI   asuI       mboII mboII
                         bmyI                          nspI        bsp1286             bsaJI  nlaIV      bpuAI earI/ksp632I
                         banII         maeIII          nspHI       bmyI  alwNI         apyI[dcm+]        bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                        sau96I
                                        nlaIV
                                        avaII
                                      mspI
                                     scrFI
                                     nciI
                                 sau3AI hpaII
                                 mboI/ndeII[dam-]
                                 dpnI[dam+]                        nlaIII
                              nlaIII    dsaV         mnlI        nspI                                     drdI  mnlI
                             rcaI       cauII      ddeI maeIII                                      mboII   ddeI
                stYI         bspHI[dam-]  asuI    eco81I       nspHI                                bpuAI  eco81I
                bsaJI      mnlI  dpnII[dam-]    bsu36I/mstII/sauI                   maeII           bbsI    bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                              aciI
                                             thaI
                                             fnuDII/mvnI
                                             bstUI
                                             bsh1236I
                                            sacII/sstII
                                            nspBII
                                            kspI
        maeII                               dsaI                                 rsaI
      rsaI                                  bsaJI                                csp6I
      csp6I                                 aciI             rsaI            maeII                          mnlI      ecoNI
     bsrI bsaAI            mnlI            fnu4HI    mnlI    csp6I          bsaAI                     hgaI     hphI    bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

      scrFI
      mvaI bsrI
      ecoRII
      dsaV
      bstNI                    rsaI          bsmAI                                                                   fnu4HI
      apyI[dcm+]               csp6I        bsaI              mnlI           taqI                                    bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9I

```
                                                  scrFI
                                                  nciI
                                                  mspI
                                                  hpaII
                                                  dsaV
                                                  cauII
                                                 xmaI/pspAI
                                                  smaI
                                                  scrFI                    scrFI
                                                  nciI                     mvaI                scrFI
                                                  dsaV                     ecoRII        mvaI
                                                  cauII                    dsaV          ecoRII
                                rsaI             fokI    mboII           bstNI               dsaV
                                csp6I         bslI  bsaJI                apyI[dcm+]          bstNI
      avaI                    bsp1407I      bslI   avaI earI/ksp632I     sexAI          bspMI apyI[dcm+]
2401  CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
      GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

           dsaI                        mspI
           bslI                     fnu4HI                                          pleI                  mnlI
           bsaJI                    bbvI hpaII                      mnlI            hinfI     nlaIV   mboII   scfI    aluI
2501  ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
      TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                       bpuAI
                                       bbsI                    nlaIII
          dsaI                        maeII                  ppu10I                                 sapI
          bsaJI                fnu4HI    xmnI mboII           nsiI/avaIII                            mboII  mnlI
        hphI             bspMI   bbvI      asp700      nlaIII     sfaNI   mnlI                       earI/ksp632I bslI
2601  GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
      CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                              styI
           scrFI                        taqI                aciI
           nciI                        salI                fnu4HI   sau96I
           mspI                     rmaI  hincII/hindII      sfiI ncoI haeIII/palI
           hpaII                    maeI  accI   scfI        haeIII/palI
           dsaV                  sau96I  pleI     pstI       eaeI  dsaI asuI                aluI
           cauII                 haeIII/palI      bsgI   aluI cfrI  bsaJI              fnu4HI
        bsmAI                    asuI    hinfI bspMI    hindIII bglI nlaIII              bbvI          maeIII
2701  CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
      GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9J

```
                                                                                                                                taqI[dam-]
                                                                                                                               claI/bsp106[dam-]
                                                                                                                              sau3AI
                                                                                                                              mboI/ndeII[dam-]
                                                                                                                              dpnI[dam+]
                                                                                                                              dpnII[dam-]
                                                                                rmaI
                    sfaNI   apoI                                               bsmI  maeI                                                nlaIII  alwI[dam-]
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

                tru9I                                                                  rsaI
              xmnI                                                                     csp6I
     sau3AI  aseI/asnI/vspI      styI                                                nlaIV
     mboI/ndeII[dam-]            ncoI                                                kpnI
     dpnI[dam+]             fnu4HI   haeI                                            hgiCI
     dpnII[dam-]            bbvI  dsaI haeIII/palI                                   banI                          aluI
    pvuI/bspCI mseI    hinPI    bsaJI                                                asp718     mnlI           pvuII
    mcrI   asp700      hhaI/cfoI nlaIII          mnlI      mnlI                     acc65I   ddeI aciI        nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                    nlaIV
                                  scrFI                                                                  scrFI
                                  mvaI                                      ppu10I                            mvaI
                                  ecoRII                                 nsiI/avaIII                   ecoRII
                                  dsaV                                   nlaIII                        dsaV
                                  bstNI                                      sphI                            bstNI
                                  apyI[dcm+]                                 nspI sfaNI                      apyI[dcm+]
                                 bsaJI                                       nspHI                           sexAI                bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

       nlaIV                              sfaNI
     scrFI                             ppu10I
     mvaI                                   nsiI/avaIII
     ecoRII                           nlaIII
     dsaV                             sphI
     bstNI                                  nspI                                                                  aciI
     apyI[dcm+]                             nspHI                                    aciI          aciI  fokI              aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9K

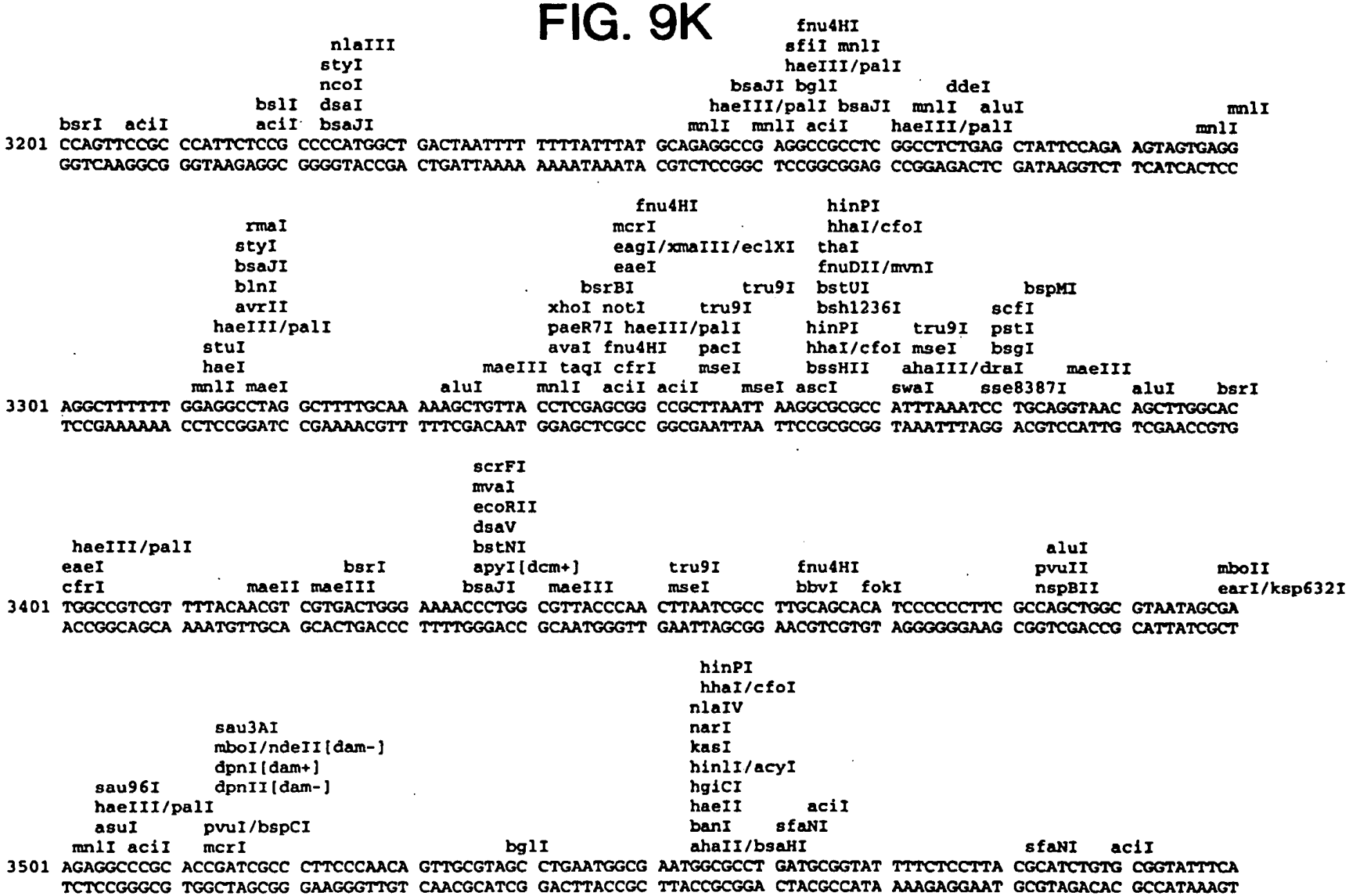

FIG. 9L

```
                                                     aciI
                 hinPI                              fnu4HI                 fnu4HI
                 hhaI/cfoI                          aciI                  hinPI
                 thaI                               thaI                  hhaI/cfoI
                 fnuDII/mvnI                        fnuDII/mvnI           thaI
                 bstUI                 hinPI        bstUI                 fnuDII/mvnI
                 bsh1236I              hhaI/cfoI hinPI                    bstUI                              hinPI
               rsaI       scfI fnu4HI    tru9I bsh1236I                   bsh1236I    aciI                   hhaI/cfoI
     aciI  maeII       csp6I    bslI  aciI     mseI hhaI/cfoI      maeIII  bbvI  maeIII                     haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

        hinPI                                                                             nlaIV
        hhaI/cfoI                                      mspI                              hgiJII
       haeII                                           hpaII                             bsp1286
     rmaI   bsrBI                                      naeI                              bmyI
     maeI   aciI          mboII                 maeII  cfr10I           aluI             banII       nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

               nlaIV                                    maeII   haeIII/palI
               hgiCI  taqI                             draIII  sau96I                                     maeII
               banI mnlI                      hphI     bsaAI   asuI                                       drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

        pleI          tru9I     pleI                              bslI
        hinfI  maeII  mseI      hinfI         bsrI               bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                 thaI                                     hgiAI/aspHI
                                                 fnuDII/mvnI                              bsp1286
                                                 bstUI                   maeII            bsiHKAI
               tru9I                          tru9I                   tru9I               bmyI    ddeI
               mseI             tru9I         mseI     apoI tru9I     mseI                apaLI/snoI  rsaI
         haeIII/palI      aluI   mseI    apoI    bsh1236I mseI    sspI  psp1406I          alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9M

```
                                                                                                             hinPI
                                                                                                             hhaI/cfoI
                                                                                                            thaI
                                                                            hinPI                           fnuDII/mvnI
                      aciI                               maeIII          fnu4HI                             bstUI
                     fnu4HI    tru9I                   maeII bsrI   nlaIII  hhaI/cfoI               nspBII  bsh1236I
                  sfaNI        mseI        aciI       bsaAI tth111I/aspI bbvI           aciI       aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                                 thaI
                                                                                                                 fnuDII/mvnI
                                                                                                                 bstUI
                                                               scrFI                                             bsh1236I
                         mspI                                  nciI                                             hinPI
                         hpaII                                 mspI                                             hhaI/cfoI
                        scrFI                             bsmAI          nspI                                  thaI
                        nciI     aciI                    esp3I hpaII fnu4HI                                    fnuDII/mvnI
                        dsaV sfaNI                      bslI   dsaV aluI nspHI                                 bstUI
       drdI             cauII fokI            aluI maeIII      cauII bbvI nlaIII    mnlI   hphI      hphI      bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                                    mnlI
                                  haeIII/palI                                                   maeII
                      mboII      sau96I                                  nlaIII                hinlI/acyI
                      bpuAI      asuI                            tru9I  rcaI                   ahaII/bsaHI
       mnlI           bbsI      eco0109I/draII                   mseI   bspHI              ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                       nlaIV
                     aciI
                    thaI
                    fnuDII/mvnI
                    bstUI                                                    rcaI
                    bsh1236I                                                 bspHI
                   hinPI                                                 bsrBI    bsmAI
                   hhaI/cfoI                                             aciI nlaIII                             sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

                  mboII                                                 fnu4HI
                  earI/ksp632I                                          aciI                        hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9N

```
                                                hgiAI/aspHI
                                                bsp1286
                                  sau3AI        bsiHKAI                                  sau3AI  nspBII     sau3AI
                                  mboI/ndeII[dam-]                                       mboI/ndeII[dam-]  mboI/ndeII[dam-]
                                  dpnI[dam+]    bmyI                                     dpnI[dam+]        dpnI[dam+]
                                  dpnII[dam-]                                        bstYI/xhoII          dpnII[dam-]
                             eco57I             apaLI/snoI                       bsrI dpnII[dam-]         alwI[dam-]
      hphI             sfaNI  mboII[dam-]       alw44I/snoI  maeIII taqI    alwI[dam-]    aciI  bstYI/xhoII                mboII
4601  CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
      GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                                   scrFI
                                                                                   nciI
                                                                 aciI              mspI
                                                                thaI               hpaII
                                                                fnuDII/mvnI        dsaV
        maeII                hgiAI/aspHI                        bstUI              cauII
       psp1406I              bsp1286  tru9I                     bsh1236I        hinlI/acyI
      xmnI                   bsiHKAI  mseI                    hinPI              hgaI                              aciI
      asp700                 bmyI     ahaIII/draI            hhaI/cfoI          ahaII/bsaHI    bcgI   mcrI  fnu4HI
4701  AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
      TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                                rsaI
                                csp6I   bsrI                                                          fnu4HI
          ddeI                  scaI hphI   maeIII      sfaNI     fokI   nlaIII                      bbvI        nlaIII
4801  CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
      GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                sau96I
                                                avaII                                                    maeIII
                                             sau3AI  asuI                                              nlaIII
                    haeIII/palI              mboI/ndeII[dam-]                                       sau3AI
                   eaeI                      dpnI[dam+]                                             mboI/ndeII[dam-]   sau3AI
                   cfrI                      dpnII[dam-]                                            dpnI[dam+]         mboI/ndeII[dam-]
                  fnu4HI                   pvuI/bspCI                                              dpnII[dam-]        dpnI[dam+]
                  aciI                     mcrI   mnlI          aluI  aciI                  nlaIII alwI[dam-]         dpnII[dam-]
4901  AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
      TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9O

```
                                                                                   hinPI
          mspI                                                                     mstI
           hpaII                                                                   aviII/fspI   bsrI
          bsaWI                                                         fnu4HI    maeII hhaI/cfoI tru9I
      nlaIV    aluI                                  maeIII      sfaNI   bbvI      psp1406I        mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                     mspI
                     hpaII                                                                 bglI
                    scrFI                                                                  sau96I
              aluI  nciI       tru9I      fokI                       sau96I                haeIII/palI
             rmaI   dsaV       mseI   bsrI       aciI                avaII      hinPI  asuI    mspI
             maeI   cauII      aseI/asnI/vspI  mnlI                  asuI       hhaI/cfoI      hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                      mspI           thaI
                      hpaII          fnuDII/mvnI              haeIII/palI
                     cfr10I          bstUI                   sau96I
                 nlaIV  hphI     bsmAI aciI         fnu4HI  nlaIV
               gsuI/bpmI        bsaI bsh1236I       bbvI bsrI asuI           mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                               ddeI
                                           sau3AI          nlaIV
        pleI                               mboI/ndeII[dam-]  mnlI
        hinfI                              dpnI[dam+]      hgiCI           tru9I
  eam1105I             fokI                dpnII[dam-]     banI            mseI       maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                   rmaI     sau3AI
                                                sau3AI hphI  mboI/ndeII[dam-]
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]   dpnI[dam+]
                                         tru9I  dpnII[dam-]  dpnII[dam-]
                     tru9I             ahaIII/draI maeI      alwI[dam-]          nlaIII               maeII
                     mseI          tru9I      bstYI/xhoII  bstYI/xhoII           rcaI               tru9I
                    ahaIII/draI    mseI mseI  alwI[dam-] mboII[dam-]             bspHI              mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9P

```
                                                  sau3AI
                                                  mboI/ndeII[dam-]
                                                  dpnI[dam+]  sau3AI                 thaI
                                                  dpnII[dam-] mboI/ndeII[dam-]
                                                bstYI/xhoII   dpnI[dam+]      fnuDII/mvnI
                                      sau3AI  alwI[dam-]    dpnII[dam-]      bstUI
                                      mboI/ndeII[dam-]      alwI[dam-]       bsh1236I
                                  hgaI                      dpnI[dam+] mboII[dam-]     hinPI       fnu4HI
                                 ddeI                       dpnII[dam-]      bstYI/xhoII hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                                  sau3AI
                                                  mboI/ndeII[dam-]
                                                  dpnI[dam+]
                                                  dpnII[dam-]
                                                alwI[dam-]
                                           aciI mspI                        bsrI          hinPI
                                  aciI  nspBII  hpaII      aluI            maeIII  eco57I  hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                  fnu4HI
                                                                                          alwNI   bbvI
                                                                                      bsrI    fnu4HI
                                                                                 maeIII       bbvI    bsrI
                                 rmaI        haeIII/palI
                                  maeI   bslI    haeI                scfi    aciI        mnlI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                                      scrFI                                   aciI                hgiAI/aspHI
                                      nciI                                 nspBII                 bsp1286
                                      mspI                          mspI    fnu4HI                 bsiHKAI
                                      hpaII                         hpaII   bbvI                   bmyI
                                      dsaV      pleI                bsaWI   hinPI mcrI             apaLI/snoI
                                      cauII     hinfI               maeIII  hhaI/cfoI              alw44I/snoI   aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9Q

```
                                                                                                      mspI
                                                             hinPI                                    hpaII
                                                             hhaI/cfoI                                bslI    fnu4HI
                           ddeI     scfI                    haeII                        aciI        bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                          scrFI
                                          mvaI          scrFI
                                          ecoRII  mvaI
                                          dsaV    ecoRII
                                          bstNI         dsaV
                        hinPI  mnlI       bsaJI         bstNI                                                 taqI
                        hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]                            mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                   haeIII/palI
                                                               haeIII/palI     scrFI
                                                             fnu4HI            mvaI
                                                             aciI              ecoRII
                                                            thaI bslI          dsaV                nlaIII
                                                            fnuDII/mvnI        bstNI bslI                  nspI
                                nlaIV                       bstUI              apyI[dcm+]    haeIII/palI  nspHI
            sfaNI             aciI                          bsh1236I       nlaIV  haeI         haeI        aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                    fnu4HI              fnu4HI
                                                                                    bbvI                bbvI pleI
                           tfiI                                           bsrBI   aciI               hinPI   hinfI
                           hinfI                  aciI          aluI      aciI  fnu4HI        mcrI   hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9R

```
                                                                    thaI
                                                                    fnuDII/mvnI
                                                                    bstUI
                                                                    bsh1236I
                                                                  hinPI
                                                                  hhaI/cfoI
                                                                 thaI
                                                                 fnuDII/mvnI
                       sapI  hinPI                               bstUI                tru9I   aluI
                       mboII hhaI/cfoI                           bsh1236I haeIII/palI       pvuII
                       earI/ksp632I                 mnlI   bslI      eaeI  tfiI aseI/asnI/vspI
           mnlI    aciI     haeII               aciI       aciI      cfrI  hinfI mseI    nspBII                  bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                        scrFI
                                                                        mvaI
                                                                        ecoRII
                                                                        dsaV
                                 tru9I                             nlaIV bstNI
                      hinPI      mseI       maeIII                 hgiCI apyI(dcm+)              mspI
       aciI           hhaI/cfoI aseI/asnI/vspI mnlI                banI bsaJI                    hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                                      tru9I
                                                                      mseI
                                                                     aseI/asnI/vspI
          aciI                                                     xmnI
        bsrBI                         aluI      nlaIII             asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
```

FIG. 10A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ec1136II
     bsp1286                                                                    thaI
     bsiHKAI                                                                    fnuDII/mvnJ
     bmyI                        rmaI    tru9I                                  bstUI
     banII                       maeI    mseI                                   bsh1236I
   taqI                          speI    aseI/asnI/vspI                 bslI    aciI maeIII
 1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
   AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
              aciI
            bglI bstNI                          maeII
           sau96I                               hinlI/acyI
           haeIII/palI     aciI                 ahaII/bsaHI
           asuI  apyI[dcm+]              aciI   aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

     maeII                                                                      maeII
    hinlI/acyI                                                                  hinlI/acyI
    ahaII/bsaHI                                  rsaI                  rsaI     ahaII/bsaHI
    aatII                              bglI      csp6I          ndeI   csp6I    aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
       aciI                                                                     nlaIII
      bglI  dsaV                                                          styI
     sau96I bstNI          rsaI                              maeII              ncoI
     haeIII/palI           csp6I                       rsaI   snaBI             dsaI hphI  aciI
     asuI   apyI[dcm+]  bsrI  nlaIII                   csp6I  bsaAI             bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 10B

```
                                                                              maeII
                                                                             hinlI/acyI               nlaIV
               rsaI                                 pleI                     ahaII/bsaHI              hgiCI
               csp6I                         aciI   hinfI             bsmAI  aatII                    banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                     aluI
                                                                                                    sstI
                                                                                                    sacI
                                                                                                    hgiJII
                                                                                                    hgiAI/aspHI
                                                                                                    ecl136II
                                                                                                    bsp1286
                                                                                                    bsiHKAI
                                                                            rsaI                    bmyI
                                   maeIII     aciI       hgaI        aciI   csp6I     mnlI          banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                             haeIII/palI
                                                                                            mcrI
                                                                                            eagI/xmaIII/eclXI
                                                                                            eaeI
                                                                                            cfrI
                                                                                           fnu4HI
                                                                                           aciI
                                                                                          thaI
                              esp3I                                                       fnuDII/mvnI
                         scrFI                                          sau96I     sacII/sstII
                         mvaI bsmAI                                          avaII       nspBII
                         ecoRII                                         asuI       kspI  scrFI
                         dsaV                                          nlaIV       dsaI  nciI
                         bstNI  hinlI/acyI                                 scrFI    bglI bslI  mspI
                         apyI[dcm+]                                        nciI   sau3AI mnlI bstUI
                    sau3AI gsuI/bpmI                                       mspI   mboI/ndeII[dam-] hpaII
                    mboI/ndeII[dam-]                                mboII  hpaII  dpnI[dam+] bsaJI dsaV
                    dpnI[dam+]  hgaI  fokI                          bpuAI  dsaV   dpnII[dam-] bsh1236I
                    dpnII[dam-] ahaII/bsaHI               mnlI      bbsI   cauII  alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
                tfiI                                                                                                  fnu4HI
              aciI                                                                                                    aciI
             thaI hinfI                                                                  bstXI                        thaI
             fnuDII/mvnI                                  aciI                        sau96I    styI               fnuDII/mvnI tru9I
             bstUI                                  maeII rsaI          pleI scfI haeIII/palI                       bstUI       mseI
             bsh1236I                          maeIII    csp6I    scfI hinfI     asuI      bsaJI                   bsh1236I   aseI/asnI/vspI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                                    sau96I
                                                                                                                    avaII
                                                                                                                    asuI
                                                                                                                  scrFI
                                                                                                                  mvaI
                                                                                                                  ecoRII
                                                                                                                  dsaV
                                                                                                                  bstNI
                                                                                                                  apyI[dcm+]
                                          maeIII                                                                  bslI bsaJI
                                         hphI   scfI          fokI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                  hinlI/acyI
                                                  ahaII/bsaHI
                                                  aatII
                                                thaI                      taqI[dam-]
                                                fnuDII/mvnI             sau3AI              bsmAI
                    aluI  taqI                  bstUI                   mboI/ndeII[dam-]    esp3I
     mnlI       hindIII                       aciI maeII                dpnI[dam+]      tth111I/aspI
     bsaJI   ddeI     claI/bsp106            hphI bsh1236I    taqI     dpnII[dam-] taqI   hgaI          sfaNI
901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
    TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                                          hinPI
      aluI              tfiI                                                                          fnu4HI
     fnu4HI             hinfI                    taqI                                               bbvI
     bbvI       mnlI  mboII                  aluI          mnlI                  aciI       aluI hhaI/cfoI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                        hinPI
                                        hhaI/cfoI     mspI
                                       thaI           hpaII
                                      aciI           mroI
                                     haeIII/palI      bspMII
                                    mcrI fnuDII/mvnI  bspEI
    sau3AI                          eagI/xmaIII/eclXI bsaWI
    mboI/ndeII(dam-)                eaeI bstUI     tfiI
    dpnI(dam+)                      cfrI bsh1236I  hinfI                  ecoRI
    dpnII(dam-)                  sfaNI  fnu4HI  bslI   accIII            apoI                          sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                        mcrI
                                                                                                        eagI/xmaIII/eclXI
                                                                                                        eaeI
                                                                                          styI          fnu4HI
       hgiAI/aspHI                                                                  thaI     ncoI       sau3AI  aciI
       bsp1286                                                                      fnuDII/mvnI         mboI/ndeII(dam-)
       bsiHKAI                                                          fnu4HI      bstUI    dsaI       dpnI(dam+)    sau3AI
       bmyI                                                             bbvI  mcrI       haeIII/palI  dpnII(dam-)   mboI/ndeII(dam-)
       apaLI/snoI                                                     scfI   mspI bsh1236I bsaJI sfaNI    fnu4HI    dpnI(dam+)
       alw44I/snoI    maeII                                 nspBII   pstI   hpaII     mnlI nlaIII   pvuI/bspCI haeIII/palI
     bslI draIII   maeIII        bspMI                     aciI     bsgI  cfr10I aciI haeI   fokI  mcrI bbvI cfrI dpnII(dam-)
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                    sau96I                                                    thaI       sau3AI
                                    avaII                                                     fnuDII/mvnI       nlaIII
                                    asuI                                                      bstUI      mboI/ndeII(dam-)
                           sau96I   rsrII/cspI                                               bsh1236I   dpnI(dam+)
                  aciI     haeIII/palI  aciI    tfiI                                        hinPI      dpnII(dam-)
     ddeI        bsrBI      asuI     cpoI       hinfI              nlaIII          ndeI hhaI/cfoI   alwI(dam-)
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                hinPI                                                    draIII
                                                hhaI/cfoI                                               nlaIV
                                               thaI                                    bsaJI            hgiCI
                                               fnuDII/mvnI                            haeIII/palI       banI
                          tth111I/aspI         bstUI                        sfaNI     sau96I           mspI
       bsrI              drdI          hgaI    bsh1236I    taqI   aluI          asuI mnlI       bslI    hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

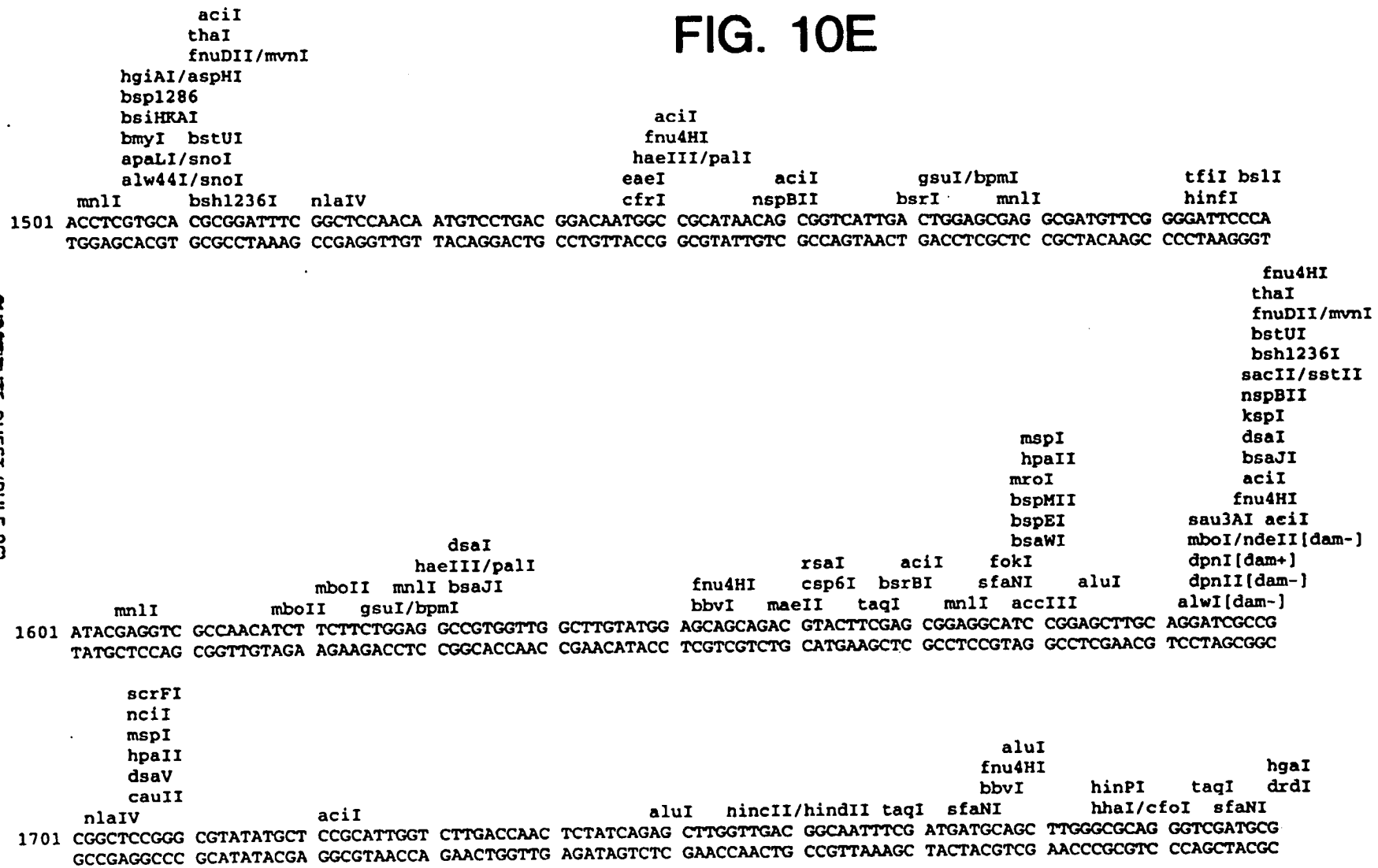
aciI
thaI
fnuDII/mvnI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI bstUI
apaLI/snoI
alw44I/snoI
aciI
fnu4HI
haeIII/palI
eaeI
cfrI
aciI
nspBII
gsuI/bpmI
bsrI
mnlI
tfiI bslI
hinfI
mnlI
bsh1236I
nlaIV
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT
fnu4HI
thaI
fnuDII/mvnI
bstUI
bsh1236I
sacII/sstII
nspBII
kspI
dsaI
bsaJI
aciI
fnu4HI
sau3AI aciI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
mspI
hpaII
mroI
bspMII
bspEI
bsaWI
dsaI
haeIII/palI
mboII mnlI bsaJI
rsaI
aciI
fokI
fnu4HI
csp6I
bsrBI
sfaNI
aluI
mnlI
mboII
gsuI/bpmI
bbvI
maeII
taqI
mnlI
accIII
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC
scrFI
nciI
mspI
hpaII
dsaV
cauII
aluI
fnu4HI
bbvI
hgaI
hinPI
taqI
drdI
nlaIV
aciI
aluI
nlaIII/hindII taqI
sfaNI
hhaI/cfoI
sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC

FIG. 10F

```
          nlaIV                                                     haeIII/palI
         mspI                                                      mcrI
         hpaII  scrFI                                              eagI/xmaIII/eclXI
         bslI   nciI                                               eaeI
        mroI    mspI                                               cfrI
        bspMII  hpaII                                             fnu4HI
        bspEI[dam-]                                               aciI
        bsaWI   dsaV                                             thaI
        accIII[dam-]                                             fnuDII/mvnI
       sau3AI   cauII                                            bstUI
       mboI/ndeII[dam-]                                          bsh1236I    sau96I               rsaI
       dpnI[dam+]                                                hinPI       avaII                csp6I
       dpnII[dam-]              rsaI                            hhaI/cfoI    asuI                scaI
       alwI[dam-]               csp6I          aciI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                         scrFI
                                                                         nciI
                                                                         mspI
                                                                         hpaII
                                                                         dsaV
                                                                        xmaI/pspAI
                                                                        smaI
                                                                        scrFI
                                                                        nciI
                                                                        dsaV
                                                                        cauII
                                                                        bsaJI
                                                                        avaI
                                                                       bsaJI
                                                                     sau3AI                          sau96I
                                                                     mboI/ndeII[dam-]       aciI     haeIII/palI
                                                                     dpnI[dam+]            fnu4HI    asuI
                                                                     dpnII[dam-]           bglI  nlaIII
                                                                     alwI[dam-]           sfiI  styI
                                                                    nlaIV cauII          eaeI   ncoI
                                                     mcrI           bstYI/xhoII          cfrI   dsaI
       hinlI/acyI                                   bslI            bamHI bsaJI ecoRI   taqI haeIII/palI
       hgaI                mnlI                                     alwI[dam-]  apoI   claI/bsp106 bsaJI
       ahaII/bsaHI        bsaJI                   sfaNI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
                    aluI
                   fnu4HI                                                                    rmaI
                   bbvI          maeIII              sfaNI   apoI                       bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                        sau3AI
                                        mboI/ndeII[dam-]
                                        dpnI[dam+]
                                        dpnII[dam-]
                                       pvuI/bspCI
                                       mcrI
                                      taqI[dam-]  tru9I                haeIII/palI
                                     claI/bsp106[dam-]                 haeI
                                    sau3AI        mseI        fnu4HI styI
                                    mboI/ndeII[dam-]          bbvI   ncoI
                                    dpnI[dam+] xmnI         hinPI    dsaI
                                    dpnII[dam-]  aseI/asnI/vspI      bsaJI
                              nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                                  nlaIV
      csp6I                                                                scrFI                          sfaNI
     nlaIV                                                                 mvaI                              ppu10I
     kpnI                                                                  ecoRII                        nsiI/avaIII
     hgiCI                                                                 dsaV                         nlaIII
     banI                          aluI                                    bstNI                             sphI
     asp718     mnlI               pvuII                                   apyI[dcm+]                        nspI
     acc65I  ddeI  aciI            nspBII                                 bsaJI                             nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                        nlaIV
                         scrFI        scrFI
                         mvaI              mvaI                              ppu10I
                         ecoRII       ecoRII                          nsiI/avaIII
                         dsaV         dsaV                           nlaIII
                         bstNI             bstNI                            sphI
                         apyI[dcm+]        apyI[dcm+]                       nspI sfaNI
                        sexAI             bsaJI                             nspHI                                aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

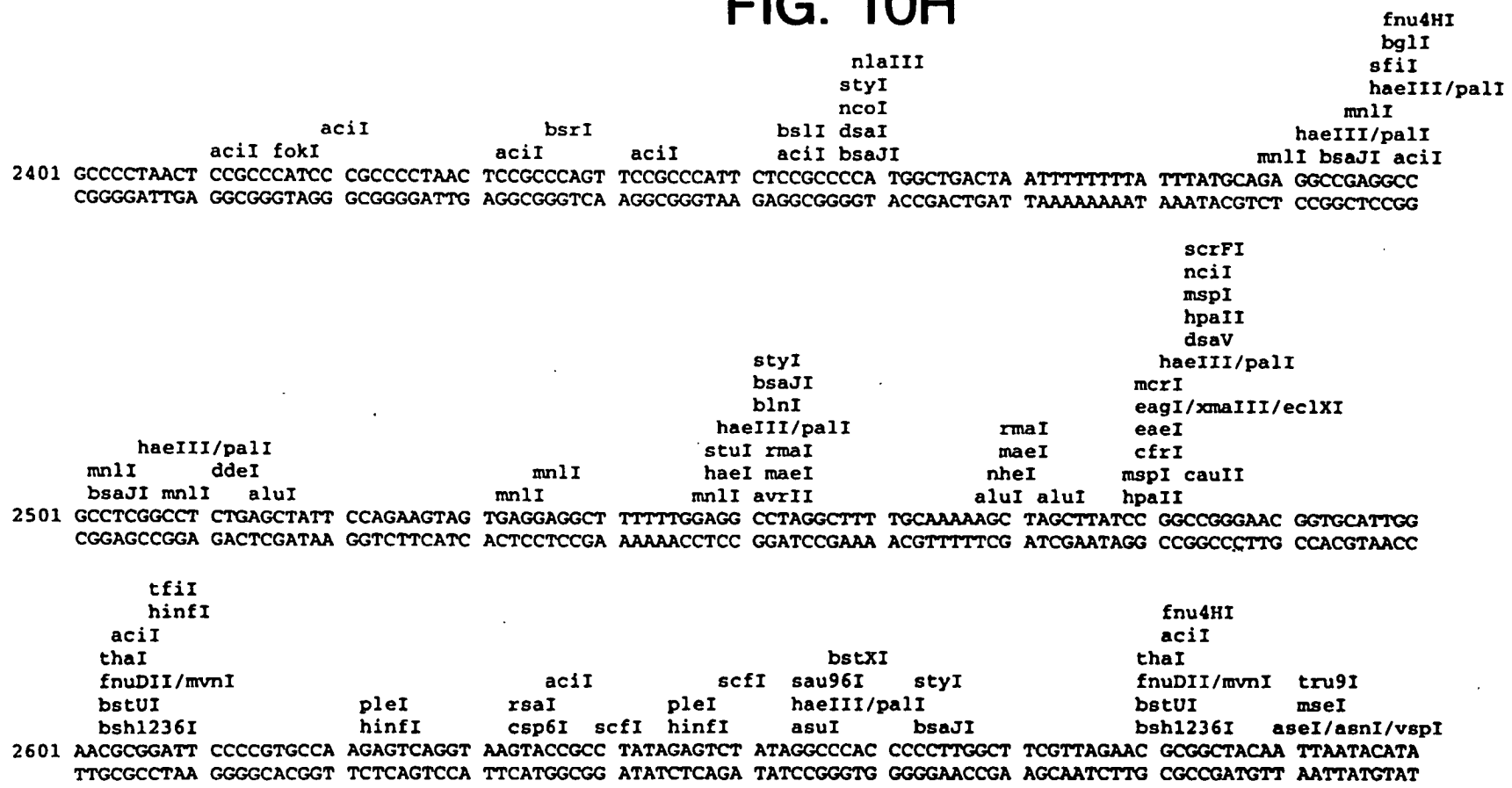

FIG. 10I

```
                sau3AI
                mboI/ndeII[dam-]
                dpnI[dam+]                                                    sau96I
                dpnII[dam-]                                                   avaII
                alwI[dam-]                                                    asuI
              taqI[dam-]                                                    scrFI
             claI/bsp106[dam-]                                              mvaI                                  rmaI
            sau3AI                                                          ecoRII              thaI    maeI
            mboI/ndeII[dam-]                                                dsaV                fnuDII/mvnI
            dpnI[dam+]                                                      bstNI                     bstUI  nheI
            dpnII[dam-]                                                     apyI[dcm+]        mnlI    bsh1236I  aluI
           alwI[dam-]                fokI                                bslI  bsaJI             bsaJI  nruI   aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                          nlaIII
                          styI
                          pflMI
                          ncoI
          sfaNI     ecoRI   dsaI                                                  rsaI              aluI
       fnu4HI taqI  apoI     bslI fokI                        rmaI            gsuI/bpmI            pvuII tth111I/aspI
       bbvI claI/bsp106      bsaJI        nlaIII  fokI       maeI            bsrI   csp6I     ecoRV nspBII   bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

          aluI
         sstI
         sacI
         hgiJII
         hgiAI/aspHI
         ecl136II
         bsp1286
         bsiHKAI
         bmyI                              hphI
         banII          mnlI               maeIII    bspMI                taqI                              bsrI
      avaI          aciI                   bstEII   hphI        bsrI      hgaI          hphI   aluI  nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

```
                                                                                                             mspI
                                                                                                             hpaII
                                                                                                             bslI
                                                       gsuI/bpmI                                            bsaWI
                                                      scrFI                                         sau3AI
                                                      mvaI                                                mboI/ndeII[dam-]
                                               haeIII/palI                                                dpnI[dam+]
                                         fnu4HI       ecoRII                                   dpnII[dam-]
           scrFI                              aciI        dsaV                                       alwI[dam-]
           mvaI                          thaI mnlI    bstNI                                         nlaIV
           ecoRII                        fnuDII/mvnI  apyI[dcm+]  pleI                              bstYI/xhoII
           dsaV                               bstUI     rsaI   pleI gsuI/bpmI                            bamHI
           bstNI      aluI                    bsh1236I  csp6I  hinfI    hinfI                            alwI[dam-]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT
           apyI[dcm+]

                                                                                      sau3AI                       styI
                                                                                      mboI/ndeII[dam-]             bsaJI
                                                                                      dpnI[dam+]                 rsaI
                                                                                      dpnII[dam-]                csp6I
                                                                                      alwI[dam-]                nlaIV
                                  fnu4HI  mboII                                      nlaIV                      kpnI
                                  bbvI    bpuAI                                      bstYI/xhoII                hgiCI
                                 scfI     bbsI                                       bamHI rsaI                 banI
                                 pstI   mspI                                       mnlI    csp6I                asp718
                                 bsgI   hpaII                                 maeIII  alwI[dam-]                acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

        sau3AI
        mboI/ndeII[dam-]                      mboII
        dpnI[dam+]            fnu4HI         bpuAI        aciI
        dpnII[dam-]           bbvI           bbsI    mboII                                     mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                                   scrFI
                                                                                   mvaI
                                                                                   ecoRII
                                                                                   dsaV
                                                                                   bstNI
                            haeIII/palI                                            apyI[dcm+]
   xmnI                    haeI      rsaI                      mnlI                bsaJI
   asp700                 mnlI       csp6I                     bslI       maeIII              maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

```
                                                                                                              sstI
                                                                                                              sacI
                                                                                                              hgiJII
                                                                                                              hgiAI/aspHI
                                                                                                              ecl136II
                                                                                                              bsp1286
                                                                                                              bsiHKAI
                                                                                                              bmyI
                                                                                                         haeIII/palI
                                        ddeI                                                            sau96I aluI
                                      celII/espI                                                        asuI  banII
                    ddeI fnu4HI       bpu1102I                                                hphI     eco0109I/draII
           scfI    mnlI   bbvI      hgaI                              accI                    maeIII   alwNI  ddeI
3401 AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG
     TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC

                                                                   sau96I
                                                                nlaIII
                                                            aciI   haeIII/palI
                                                          fnu4HI   asuI
                                                          bglI styI
                                                  aluI    sfiI  ncoI
                                                hindIII  eaeI  dsaI                       aluI
                                               tru9I     cfrI  bsaJI                     fnu4HI
        maeIII    aluI                         mseI    taqI haeIII/palI                   bbvI          maeIII
3501 CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
     GAGCGGGCAG TGTTTCTCGA AGTTGTCCCC TCTCACAATT CGAAGCTACC GGCGGTACCG GGTTGAACAA ATAACGTCGA ATATTACCAA TGTTTATTTC

                                                                                                              sau3AI
                                                                                                              mboI/ndeII[dam-]
                                                                                                              dpnI[dam+]
                                                                                                              dpnII[dam-]
                                                                                                             pvuI/bspCI
                                                                                                             mcrI
                                                                                                            taqI[dam-]
                                                                                                           claI/bsp106[dam-]
                                                                                                          sau3AI
                                                                                                          mboI/ndeII[dam-]
                                                                                                          dpnI[dam+]
                                                         rmaI                                             dpnII[dam-]
      sfaNI    apoI                                  bsmI maeI                                nlaIII    alwI[dam-]
3601 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCGAT
     GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA GACCTAGCTA
```

FIG. 10L

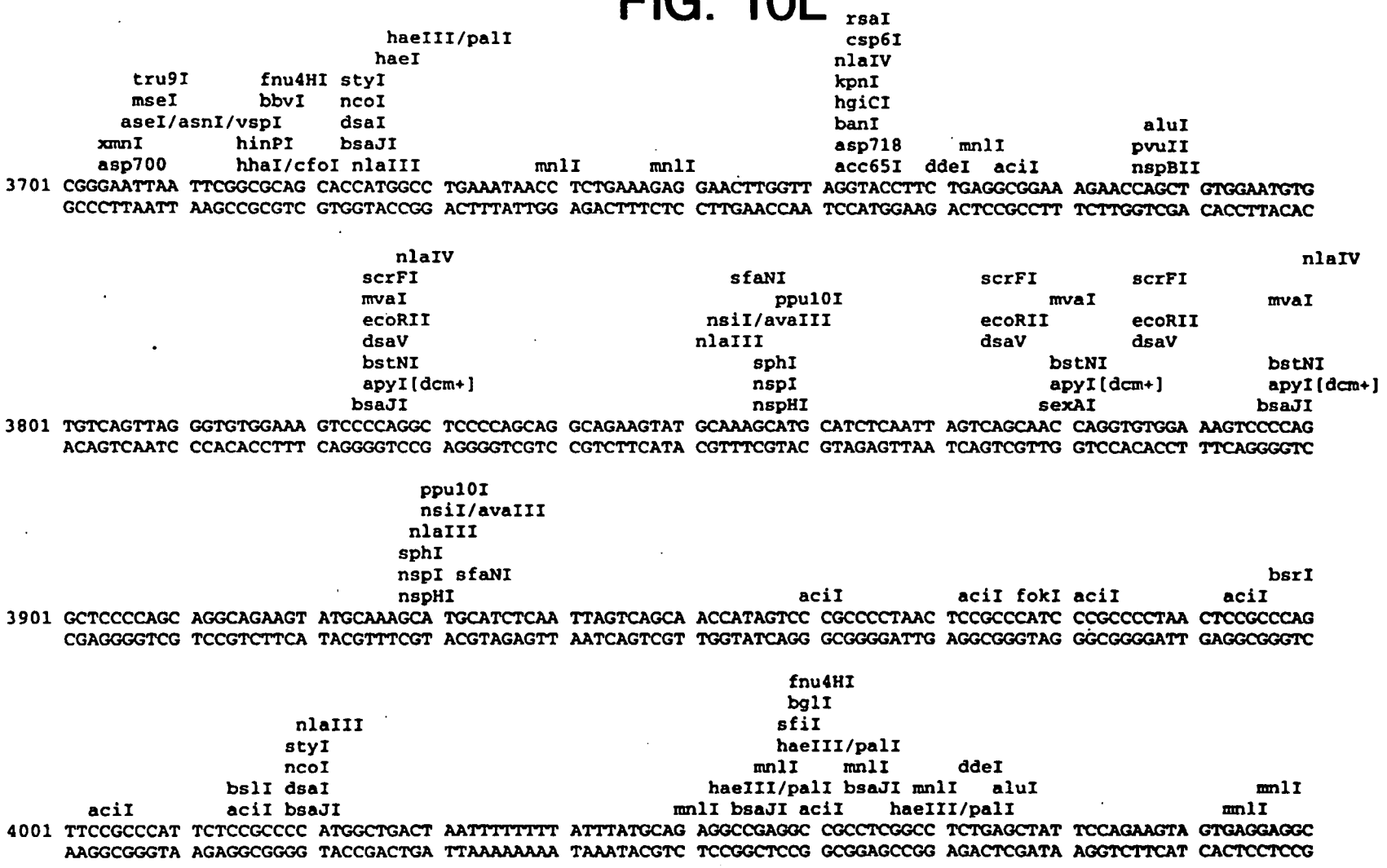

FIG. 10M

```
                styI                                                                                                scrFI
                bsaJI                                                                                               mvaI
                blnI                                                                                                ecoRII
             haeIII/palI                  tru9I              haeIII/palI                                         dsaV
            stuI rmaI                     mseI               eaeI                                                bstNI
            haeI maeI                    hpaI  aluI         cfrI                      maeIII                    apyI[dcm+]        tru9I
           mnlI avrII                 aluI hincII/hindII  bsrI                  maeII    bsrI               bsaJI  maeIII     mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                                  sau3AI
                                                                                  mboI/ndeII[dam-]
                                                                 sau96I           dpnI[dam+]
                                                                 haeIII/palI
                                                aluI             mnlI  aciI       dpnII[dam-]
                fnu4HI                         pvuII            mboII asuI       pvuI/bspCI
                bbvI  fokI                     nspBII           earI/ksp632I     mcrI                              bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

      hinPI
      hhaI/cfoI
     nlaIV                                                                                                        hinPI
     narI                                                                                                         hhaI/cfoI
     kasI                                                                                                        thaI
     hinlI/acyI                                                                                                  fnuDII/mvnI                    hinPI
     hgiCI                                                                                                       bstUI              hinPI       hhaI/cfoI
     haeII      aciI                                                                                             bsh1236I           hhaI/cfoI
     banI   sfaNI                                                                                          rsaI       scfI fnu4HI    tru9I
     ahaII/bsaHI                          sfaNI    aciI            aciI  maeII                             csp6I   bslI   aciI     mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                            fnu4HI
        aciI                hinPI
      fnu4HI                hhaI/cfoI                              hinPI
      aciI                 thaI                                   hhaI/cfoI
     thaI                  fnuDII/mvnI                           haeII
     fnuDII/mvnI           bstUI                              hinPI rmaI
     bstUI                 bsh1236I     aciI                  hhaI/cfoI    bsrBI
     bsh1236I         maeIII  bbvI maeIII                    haeII  maeI   aciI              mboII                    maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                    nlaIV
     mspI                           hgiJII                                  mnlI
     hpaII                          bsp1286                             nlaIV
    naeI                            bmyI                                hgiCI
    cfr10I            aluI          banII          nlaIV                banI    taqI                    hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

        maeII    haeIII/palI
       draIII    sau96I                                  maeII  pleI          tru9I     pleI
       bsaAI     asuI                                    drdI    hinfI maeII   mseI      hinfI          bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                      thaI
                                                                                                      fnuDII/mvnI
                                                                                                      bstUI
                                                                                tru9I                 bsh1236I
          bslI                                                                  mseI          tru9I       tru9I
         bslI   avaI                                                haeIII/palI          aluI    mseI   apoI mseI   apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                    hgiAI/aspHI
                                    bsp1286
                      maeII         bsiHKAI                                                                 tth111I/aspI
                    tru9I           bmyI    ddeI                      aciI                                  maeIII
        tru9I       mseI            apaLI/snoI  rsaI                fnu4HI    tru9I                       maeII
        mseI    sspI  psp1406I      alw44I/snoI csp6I          sfaNI         mseI        aciI        bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                        hinPI
                                        hhaI/cfoI                 mspI
                                    thaI                          hpaII
                hinPI                   fnuDII/mvnI               scrFI                                   bsmAI
            fnu4HI                      bstUI                     nciI     aciI                           esp3I
            bbvI                    nspBII  bsh1236I              dsaV sfaNI                            bslI
       nlaIII  hhaI/cfoI    aciI       aciI  hgaI       drdI       cauII fokI              aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 10O

```
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                                                                                bstUI
                                                                                                                bsh1236I
      scrFI                                                                                                    hinPI
      nciI                                                                                                     hhaI/cfoI                                                  mnlI
      mspI      nspI                                                                                           thaI                                                      haeIII/palI
      hpaII fnu4HI                                                                                             fnuDII/mvnI                    mboII                      sau96I
      dsaV aluI nspHI                                                                                          bstUI mnlI                     bpuAI                      asuI
      cauII bbvI nlaIII     mnlI    hphI       hphI                                                          bsh1236I                       bbsI                      eco0109I/draII
5001  TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
      AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                                                                                                                nlaIV
                                                                                                                                                               aciI
                                                                                                                                                               thaI
                                                                                                                                                               fnuDII/mvnI
                                                                                maeII                                                                          bstUI
                 nlaIII                                                         hinlI/acyI                                                                     bsh1236I
               tru9I rcaI                                                       ahaII/bsaHI                                                                   hinPI
               mseI  bspHI                                                ddeI aatII                                                                          hhaI/cfoI
5101  AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
      TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG

                        rcaI
                        bspHI
                      bsrBI   bsmAI                                                                                                 mboII
                      aciI nlaIII                                                  sspI                                             earI/ksp632I
5201  AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
      TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                                                                                                        hgiAI/aspHI
                                                                                                                                                                        bsp1286
                                                                                                                                                  sau3AI                bsiHKAI
                                                                                                                                                  mboI/ndeII[dam-]
                                                                                                                                                  dpnI[dam+]            bmyI
                                                                                                                                                  dpnII[dam-]
                    fnu4HI                                                                                                                  eco57I                apaLI/snoI
                    aciI                                                hphI                    hphI                                  sfaNI mboII[dam-]     alw44I/snoI  maeIII
5301  CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
      GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
          sau3AI                                                                                                  aciI
          mboI/ndeII[dam-]   sau3AI                                                                              thaI
          dpnI[dam+]         mboI/ndeII[dam-]                                                                    fnuDII/mvnI
          dpnII[dam-]        dpnI[dam+]                psp1406I        hgiAI/aspHI                               bstUI
         LstYI/xhoII         dpnII[dam-]               xmnI            bsp1286  tru9I                           bsh1236I
      bsrI          nspBII   alwI[dam-]                asp700          bsiHKAI  mseI                           hinPI
   taqI   alwI[dam-]  aciI   bstYI/xhoII             mboII maeII       bmyI     ahaIII/draI                    hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                    scrFI
                    nciI
                    mspI
                    hpaII
                    dsaV
                    cauII
                hinlI/acyI                                                          rsaI
                hgaI                          aciI                                  csp6I  bsrI
                ahaII/bsaHI    bcgI   mcrI fnu4HI          ddeI                   scaI hphI   maeIII      sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                          sau96I
                                                                                                          avaII
                                                                                                sau3AI  asuI
                                                                  haeIII/palI                   mboI/ndeII[dam-]
                                                                  eaeI                          dpnI[dam+]
                                                                  cfrI                          dpnII[dam-]
                                        fnu4HI                    fnu4HI                        pvuI/bspCI
      fokI  nlaIII                      bbvI        nlaIII        aciI                          mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                    nlaIII
                                sau3AI maeIII                   mspI
                                mboI/ndeII[dam-]    sau3AI   nlaIV
                                dpnI[dam+]          mboI/ndeII[dam-]
                               alwI[dam-]           dpnI[dam+]   hpaII
   aluI  aciI              nlaIII dpnII[dam-]       dpnII[dam-] bsaWI aluI                                  maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

```
                                                                                   mspI
                             hinPI                                                 hpaII
                           mstI                                                   scrFI
                           aviII/fspI      bsrI                        aluI ncII          tru9I     fokI
          fnu4HI         maeII hhaI/cfoI  tru9I                      rmaI  dsaV           mseI   bsrI       aciI
     sfaNI  bbvI        psp1406I          mseI                       maeI  cauII        aseI/asnI/vspI   mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
     GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC

                                                                                                  aciI
                             bglI                                             mspI               thaI
                           sau96I                                             hpaII              fnuDII/mvnI
            sau96I         haeIII/palI                                      cfr10I               bstUI
            avaII      hinPI asuI    mspI                                 nlaIV  hphI          bsmAI        fnu4HI
            asuI       hhaI/cfoI     hpaII                              gsuI/bpmI              bsaI bsh1236I bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
     CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC

                                                                                                        ddeI
         sau96I                                                                                      sau3AI
         asuI                                                 pleI                                   mboI/ndeII[dam-]
        nlaIV                                                 hinfI                                  dpnI[dam+]
     bsrI haeIII/palI       mnlI                          eam1105I             fokI                  dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
     GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA

                                                                                                              hphI
                                                                                                            rmaI
                                                                                                         sau3AI
                                                                                                         mboI/ndeII[dam-]
                                                                                                         dpnI[dam+]
      mnlI                                                                                     tru9I    dpnII[dam-]
     nlaIV                                                                      tru9I        ahaIII/draI maeI
     hgiCI        tru9I                                                         mseI       tru9I      bstYI/xhoII
     banI         mseI       maeIII                                          ahaIII/draI   mseI mseI  alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
     TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC
```

FIG. 10R

```
                                                                                                     sau3AI
                                                                                                     mboI/ndeII[dam-]
     sau3AI                                                                                          dpnI[dam+]
     mboI/ndeII[dam-]                                                                                dpnII[dam-]
     dpnI[dam+]                                                                                     bstYI/xhoII
     dpnII[dam-]                                                                             sau3AI  alwI[dam-]
     alwI[dam-]           nlaIII              maeII                                          mboI/ndeII[dam-]
    bstYI/xhoII          rcaI             tru9I                                              dpnI[dam+] mboII[dam-]
   mboII[dam-]           bspHI            mseI                       ddeI hgaI               dpnII[dam-]
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA

                   thaI                                                                 sau3AI
   sau3AI          fnuDII/mvnI                                                          mboI/ndeII[dam-]
   mboI/ndeII[dam-]                                                                     dpnI[dam+]
   dpnI[dam+]      bstUI                                                                dpnII[dam-]
   dpnII[dam-]     bsh1236I                                                            alwI[dam-]
   alwI[dam-]     hinPI       fnu4HI                                 aciI              mspI
  bstYI/xhoII     hhaI/cfoI   bbvI                            aciI  nspBII             hpaII      aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

           bsrI         hinPI                    rmaI                haeIII/palI
       maeIII    eco57I      hhaI/cfoI                maeI      bslI     haeI                     scfI     aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                     scrFI
                                                                     nciI
                                     fnu4HI                          mspI                           mspI
                              alwNI  bbvI                            hpaII                          hpaII
                             bsrI   fnu4HI                           dsaV      pleI               bsaWI      hinPI
    mnlI                  maeIII    bbvI     bsrI                    cauII     hinfI            maeIII       hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                              hgiAI/aspHI
    mcrI                      bsp1286
   aciI                       bsiHKAI
  nspBII                      bmyI                                                                        hinPI
 fnu4HI                       apaLI/snoI                                                                  hhaI/cfoI
 bbvI                         alw44I/snoI  aluI                          ddeI      scfI                  haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

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                                                                                               scrFI
                                                                                               mvaI          scrFI
                                                                                               ecoRII  mvaI
                                             mspI                                              dsaV    ecoRII
                                             hpaII                                             bstNI         dsaV
                                             bslI    fnu4HI                        hinPI  mnlI bsaJI         bstNI
                                aciI        bsaWI    aciI                          hhaI/cfoI    aluI apyI[dcm+]    apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                 fnu4HI
                                                                                                                 aciI
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                     taqI                              nlaIV                    bstUI
                                    mnlI  drdI    hgaI           sfaNI               aciI                       bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                         haeIII/palI
                      scrFI
                      mvaI
                      ecoRII
                      dsaV                    nlaIII
                      bstNI bslI                   nspI
        bslI          apyI[dcm+]      haeIII/palI  nspHI                       tfiI
      haeIII/palI nlaIV haeI       haeI            aflIII                      hinfI                    aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

```
                                                                                                                               thaI
                                                                                                                               fnoDII/mvnI
                                                                                                                               bstUI
                                                                                                                               bsh1236I
                                                                                                                              hinPI
                                                                                                                              hhaI/cfoI
                                                                                           hinPI                             thaI
                   fnu4HI                fnu4HI                                       sapI hhaI/cfoI                         fnuDII/mvnI
                   bbvI                  bbvI  pleI                                   mboII                                  bstUI
          bsrBI  aciI                 hinPI  hinfI                                    earI/ksp632I                           bsh1236I
    aluI  aciI  fnu4HI      mcrI      hhaI/cfoI            mnlI     aciI   haeII                      mnlI         bslI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                 tru9I    aluI
     haeIII/palI          pvuII                                                           tru9I                      nlaIV
    eaeI tfiI    mseI     nspBII                                             hinPI        mseI      maeIII          hgiCI
    cfrI hinfI aseI/asnI/vspI              bsrI       aciI                   hhaI/cfoI  aseI/asnI/vspI mnlI         banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

     scrFI
     mvaI
     ecoRII
     dsaV
     bstNI
     apyI[dcm+]             mspI                           aciI                                                     xmnI
    bsaJI                   hpaII                        bsrBI                               aluI       nlaIII     asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCI/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1995	08.12.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21

Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21

Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21

Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21

A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21

A	WO,A,90 12025 (UNIV LELAND STANFORD JUNIOR) 18 October 1990 cited in the application see the whole document	1-21

A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21

A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document	1-4

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

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		JP-A-	6040942	15-02-94
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